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PRELIMINARY NOTE ON THE ACTIVATION OF THE EGG IN THE CRUCIAN CARP (*Carassius auratus*).

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1) Introduction

The so-called activation of the unfertilized egg is the first step of the change in the process of fertilization.

This phenomenon is brought about before the entrance of the spermatozoa by the breakdown of cortical alveoli which are evenly embedded in the cortical protoplasm of the unfertilized egg. The breakdown of the cortical alveoli and the subsequent elevation of the chorion can also be induced by picking, heating, electric current and chemical reagents such as sodium taurochorate, sodium glycochorate, sodium oleate, saponin and digitonin etc. (Yamamoto '39, '44, '47). Thus the egg has an activable system in itself which causes the cortical change.

Yamamoto ('49) worked on the same subject in detail in the oryzias egg. Having found minute cortical granules embedded in the surrounding cortical alveoli, he suggests that an esterase-like substance in the granules may participate in the activating reaction.

Ishida ('49, '50) thought a kind of esterase decomposed the cortical lipoid of the egg at the time of activation, and the negative wave caused by the ferment may be absorbed in the plasma surface of the egg in an instant.

As in the above eggs, the cortical alveoli are also observed in the unfertilized egg of the crucian carp.

The present study deals with some experiments on the response of the activating and inhibiting enzymes in the activation. The existence of the lipase (esterase) in the egg is to be confirmed. Though it may be considered unnatural to apply the enzyme contained in the pancreas, stomach, milk etc. directly to the egg as an activator or inhibitor, a series of experiments have been carried out with these enzymes and the following results obtained.

2) The response of the cortical alveoli to the esterase activator.

The ripe unfertilized egg of the crucian carp looks less transparent than that

of the oryzias. When the egg is immersed in water, the cortical alveoli disappear in 13~15 seconds and the perivitellin space appears in 2~3 minutes. The ripe egg of the crucian carp loses its fertilizability within 40 seconds in fresh water. Among various esterase activators, lead-acetate gave good results in the experiment.

When the ripe egg is treated with 0.01% Ringer-lead-acetate solution before the fertilization and returned to isotonic Ringer's solution (M/7.5 NaCl 100 parts + M/7.5 KCl 2 parts + M/11 $CaCl_2$ 2.1 parts, buffered Ph. 7.0), the breakdown of the cortical alveoli and subsequent elevation of the chorion ensue. The breakdown of the cortical alveoli is also caused by 0.005% Ringer-pancreatin solution. However, if the ripe egg is immersed in this solution for 15~18 minutes, the chorion is partly dissolved out.

3) The response of the cortical alveoli to esterase inhibitor.

Physiologically, the response of the cortical alveoli to esterase inhibitor is of more interest than their response to an activator.

The breakdown of the cortical alveoli of the ripe unfertilized egg occurs just at the moment when the egg is treated with distilled water. However, when the egg is immersed in 0.01% Ringer-monoiodo-acetate solution for 5 minutes, then washed with isotonic Ringes's solution and transferred to distilled water, the breakdown of the cortical alveoli does not occur until 60~90 seconds have elapsed.

The effect of the solution of 0.1% Ringer-lead-phosphate is the same as that of the above solution. If the ripe unfertilized egg is first immersed in 0.01% Ringer-monoiodo-acetate, then washed with isotonic Ringer's solution and finally immersed in 0.01% Ringer-lead-acetate, the breakdown of the cortical alveoli occurs after 5~6 minutes and the elevation of chorion ensues.

The same result is also obtained by using lead-phosphate instead of monoiodo-acetate.

4) The response of the cortical alveoli to the decomposed substance of the eaterase.

As the decomposed substances of the esterase (especially, lipase and lecithase), fatty acid, alcohol, cholin, lysolecithin, phosphoric acid, glycerophosphoric acid and glycerin are produced.

Of these substances, fatty acid (stearic acid and oleic acid), alcohol, lysolecithin, phosphoric acid and glycerophosphoric acid are examined. Cholin which is physiologically important, was not examined in this experiment. Lysolecithin

was prepared from fresh hen's egg by means of Nikuni's method (Nikuni '32).

An activation did not occur in such solutions as 0.1% Ringer-alcohol, 0.01% Ringer-phosphoric acid and 0.01% Ringer-glycerophosphoric acid. The solution of 0.003% Ringer-lysolecithin has also no effect. The lack of response of the cortical alveoli to the lysolecithin may be accounted for by the low concentration of the solution. However, further detailed research is desirable.

Of fatty acids which affect the egg, oleic acid and stearic acid were used. When the egg is immersed in 0.01% Ringer-oleic acid solution for 2~3 minutes and returned to isotonic Ringer's solution, the cortical alveoli disappear and subsequently the perivitellin space is formed.

On the other hand, if the ripe unfertilized egg is immersed directly in 1% oleic acid solution for 60~90 seconds, the egg shows no change. However, if the same is returned to isotonic Ringer's solution after washing several times with solution, the breakdown of the cortical alveoli takes place subsequently.

The same result is also obtained with stearic acid. The effect of stearic acid on the egg is stronger than that of oleic acid, as the egg immersed in 1% stearic acid solution for more than 40 seconds, shows cytolysis.

5) The lipase (esterase) of the egg.

The lipase (esterase) from the egg is obtained by modified Rona and Michaelis method ('11, '24).

On account of the immature stage of the ovary of the crucian carp, satisfactory preparation of the enzyme was not possible in this experiment.

The enzyme preparation is as follows ;

Activated egg (10 minutes immersion in distilled water)
↓
Grind in a mortar and mix with four to five time volume of M/7.5 NaCl solution and then kept for 12~16 hrs. at room temperature.
↓
Centrifuge
→ Filtrate | Precipitation

Filtrate
↓
Added with 6/10 saturated ammonium sulphate
↓
Centrifuge
→ Filtrate | Precipitation

Precipitation
↓ Dialyse
Dissolve in four to five time its volume of glycerin - - - - - *Enzyme solution*

As the substrate, saturated tributylin was used, added with sodium oleate, calcium chloride, albumin and buffer solution (Ammonium buffer, Ph. 8.5). The mixture was kept at a temperature of 37°C.

The activity of the enzyme was measured by the number of falling drops using the Stalagmometer. The number of drops every 20 minutes is as follows.

Time	At the start	After 20 minutes	After 40 minutes	After 60 minutes
number of drops	57	55	53	51

This is approximately 0 25 butylase units.

From the above results, it has been concluded that some esterase-like substance is contained in the egg.

It is a further question whether the esterase-like substance participates in the activation processes or not. However, taking into consideration Yamamoto's detailed observation as well as Ishida's results ('49), it is highly probable that the esterase-like substance does have connection with activating process.

The author wishes to express his thanks to Professor Dr. T. Inukai for his guidance; he is also indebted to the Fisheries Association in Ōnuma for allowing the collection of the samples used in this work.

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（水產科學研究所業績　第86號）

STUDIES ON BOUND WATER IN FISH MEAT MUSCLE (II)

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3. On the molecular theoretical Bound Water in fish meat protein and fish meat by the vapour tension method.

As stated in the previous paper, Sponsler, Bath and Ellis[3d] have discussed the molecular constitution of gelatine-gel on the basis of the relation between analytical results by X-ray diffraction of gelatine and the analytical results of constituent amino acids of gelatine. They have concluded that the so-called Bound Water in the molecule of water binds with hydrogen centers which are capable of forming hydrogen bonds in gelatine molecule, and the amount of the Bound Water depends in chemical equivalency on the number of molecules of gelatine.

Lloyd,[4b,c] Pauling[3b], Buswell[3c] and Higashi et al.[1] have independently been establishing the definition of the so-called molecular theoretical Bound Water on the basis of the hydrogen bond formation as Sponsler et al., stated.

Recently as to the adsorption of gases by catalysers, in proportion to the active engagement in analytical study of the adsorption isotherm by the theory of multi-molecular layers adsorption which was offered by Brunauer et al.[32a], this theory has been applied to the study of the adsorbed water in gelatine and other various protein.[31][42c, d]

One of the typical investigators, Bull[31] has said that the surface of the solid molecule of protein consists of hydrophilic planes, and that the molecules of water are adsorbed among the planes.

The present author has analyzed the thermal change in the water content "g" – water-activity "a" curve obtained by vapour tension method by using gelatine, fish meat protein and raw fish meat and has analyzed the same curve by the equation of B. E. T. of Brunauer et al. in order to learn whether the molecular theoretical Bound Water of protein is the molecule of water combined with the molecule of protein through the hydrogen bond formation in a definite combining potential.

These experiments have been carried out at the same time with those described in Experiment II, I[3a] of the previous paper, so there are few overlapping results

in regard to the gelatine and raw fish meat; the author has avoided those overlapping results.

(1) Experimental Methods.

(I) Samples.

(A) Gelatine.

The samples of gelatine in these experiments were the same as in Experiment III, 1, of the previous paper, gelatine No. 2 and No. 3. The sample of No. 2 gelatine was purchased on the market (water content 17.95%, ash 0.69%) and the sample of No. 3 was some purified by the Second Faculty of Engineering of Tokyo University (water content 18.66%, ash 0.43%).

In order to clarify the relation between salts and Bound Water, the author has added NaCl (Japanese Pharmacopeia) in proportion of 0.3 and 25% to the dried matter of sample of gelatine No. 2. The author has used sample of gelatine No. 2 to which cobaltous chloride in the proportion of 27% was added for the dried matter of the gelatine according to Hatschek's method.

To 1∼3 gm of these samples of gelatine was added distilled water in a definite volume; they were let sufficiently swell in room temperature (18°∼20°C), and then dissolved by heat on the water bath. After cooling, the solidified samples become gelatine-gel containing 80∼90% of water. The author has used these gelatine gel samples crushed in the agate mortar.

(B) Fish Meat Protein

Two kinds of fish meat protein were used, one of them was prepared from the common Squid (*Ommastrephes sloani pacificus*) meat which was soluble in 0.8% NaCl solution, and the other was prepared from the Cod fish (*Gadus macrocephalus*) meat which was soluble in 0.2% NaOH solution. These proteins were prepared carefully in the laboratory of Prof. Yoshimura in the Faculty of Fisheries (Hakodate), Hokkaido University. The former was prepared by the following method. To the crushed raw fresh Squid meat was added 10 times volume of 0.8% NaCl solution; after 30 minutes shaking, it was placed in ice room for 12 hours, then ammonium sulphate was added to the solution in half saturation. The precipitated protein was separated by the centrifugal separator and then the protein was dialized several times with distilled water, and washed with alcohol and ether. The washed protein was dried at low temperature. The main component of this protein was myosin.

The latter fish meat protein was prepared by the following method. The crushed Cod fish meat was extracted by 10 times volume of 0.8% NaCl solution

for 12 hours. The extracted solution was separated by the centrifugal separator, and the insoluble residue was again extracted with five times of 0.2% NaOH solution for 12 hours. After centrifugal separation, 2-N acetic acid was added to the extracted solution to make the pH value 5.5. The precipitated protein was filtered and the residue was washed with alcohol and ether, and then dried. The main component of this protein was myotelin.

These two kinds of fish meat protein were not purified proteins containing only a single component, but they may have several kinds of protein in their components. The author has used these protein for a first preliminary test to compare with the sample of gelatine in order to study Bound Water in the marine animal meat protein. In the present experiment, to 2～3 gm of the sample of protein was added an adequate volume of distilled water and then the sample was allowed to absorb water sufficiently to make up an 80～90% water content. At the time of the experiment with the NaCl added protein, to the dried protein was added the calculated amount of NaCl (J. P.) in the percentage of weight as well as the sample of gelatine added with NaCl.

(C) Fish Meat Sample.

In order to compare the results obtained from the fish meat protein with the result from the raw fresh Atka Mackerel meat (*Pleurogrammus azonas JORDAN et METZ*) at 15°C in Experiment Ⅲ, 1, the author has carried out the experiment at 10°C using raw Atka Mackerel meat

(Ⅱ) Experimental Method.

(A) Vapour tension method.

The method of determining vapour tension of water in the sample was the same as that described in the Experiment Ⅲ, 1, in the previous paper.[38a]

The temperature of the determination was 13°C and 8° (±1°C) for the samples of gelatine, and 15° and 10° (±1°C) for the samples of fish meat protein and raw fresh fish meat.

After the determination of the vapour tension of the samples at their every water-content, the weights of the samples were once estimated. After the last determination of the vapour tension of the samples, they were dried in the drier at 105°～110°C, and the constant weight of every sample was determined by the usual method. From those data, the values of the water-content of every sample were reckoned at each time of the estimation of the vapour tension.

The method of determining the vapour tension employed by the author is supposed the best method for the easily denaturizable and decomposable samples

such as the protein or the living matter, because the dehydrating velocity is high and the dehydration is done at comparatively low temperature. The dehydration from the sample is done in a definite time at a definite lower temperature by the rotary oil vacuum pump.

But on the other hand, that there is certain fault in the method yielding some error is supposed in the estimation of the water-content. However, as this method gives values the same as those obtained by the usual vapour tension method using adsorbents (or desiccating agents) such as sulphuric acid[1(a, b)], calcium chloride or sodium bromide[39] taking a long time, the author has thought this method sufficiently applicable to determination of the amount of water in the samples.

(B) Electrical resistance method.

The values from the method by electrical resistance for the samples of No. 2 and No. 3 gelatine and the samples of No 2 gelatine to which NaCl was added in the proportion of 0.3 and 25% respectively to the dried matter were obtained in the same way as described in Experiment Ⅱ. 1, of the previous paper. The electrodes used in this experiment were made of brass 0.7 cm. in length and 2 mm. in diameter; the distance between the two electrodes was 2.3 cm. Estimates were made at a temperature of 18°C. Electric current was 50 cycles A. C. The type of electrical resistance was Wheatston's bridge.

(Ⅲ) Arrangement of the experimental results.

As in Experiment Ⅱ, 1, described in the preceding paper[38], the water-content "g" — water-activity "a" curve (strictly speaking, water-content "g" — relative vapour pressure "p/p_o" curve) assumes a S-shape as a characteristic.

The part of A of the curve where the range is 0.1～0.7 of the values of "a" (or p/p_o) and the part of C where the range is 0.2～0.0 of the same value, are steep grade. The part of B where the range is 0.7～0.2 of the same value is a gentle grade. In order to examine in detail the properties of water at the parts of B and C, the author has examined the thermodynamic properties of water in the samples and analyzed the curves above obtained by B. E. T. equation.

(A) Calculations of the change of values of differential molal free energy, ΔF, differential molal enthalpy, ΔH, and differential molal entropy, ΔS, at every water-contents of the samples.

The water-content "g" — water-activity "a" curves obtained at the definite temperatures by the vapour tension method in this experiment perhaps indicate the dehydrating process of so-called adsorbing isotherm which shows the relation of adsorbed amount of water in the samples and the water-activities at the time

when the values of adsorbed amount were given.

Therefore, when one mol of water molecule is adsorbed to an indefinite amount of samples at various water-contents of the samples, the change of value of differential molal free energy is calculated by the following equation (3)[33, 40]:

$$\bar{F}^{\circ}-\bar{F}=\Delta\bar{F}=-RT\ln p/p_0=-RT\ln a \quad \text{--------} \quad (3)$$

where, "$\bar{F}^{\circ}$" is a differential molal free energy when one mol of water is present in the pure phase (namely pure water) at the temperature of T°K. "$\bar{F}$" is differential molal free energy when one mol of water is present in the sample at the same temperature. "R" is gas constant, "p_0" and "p" are saturated pressures of pure water and of water in the sample at the temperature of T°K.

If the water in the samples conforms to the rule of true solution, "p/p_0" will be shown as the water-activity "a" in the sample at saturated pressure "p".

The change of the value of differential molal enthalpy $\Delta\bar{H}$ per one mol of water at the adsorption of water is calculated by the following equation (4)[33, 40]:

$$\Delta\bar{H}=-T^2\frac{d}{dT}\left(\frac{\Delta\bar{F}}{T}\right)=-\frac{RT_1T_2}{T_1-T_2}\left(\ln\frac{p_2}{p_{02}}-\ln\frac{p_1}{p_{01}}\right)=-\frac{RT_1T_2}{T_1-T_2}\ln\frac{a_2}{a_1} \quad \text{--} \quad (4)$$

where, "p_i" and "p_{0i}" are the saturated pressures of water in the sample and of pure water respectively at the temperature of T°_iK. "a_i" is the water-activity of water in the sample at T°_iK.

If $\Delta\bar{S}$ is the change of value of differential molal entropy per one mol of water as well as $\Delta\bar{F}$ and $\Delta\bar{H}$ at the time of adsorption of water in the sample at T° K, there is surely a thermodynamic functional relation among those values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ as in the following equation (5):

$$\Delta\bar{F}=\Delta\bar{H}-T\Delta\bar{S} \quad \text{--------------------} \quad (5)$$

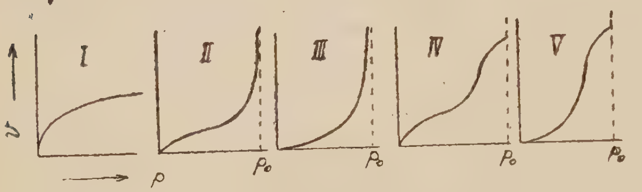
When one wishes to calculate the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$, one must strictly give consideration of hysteresis and capillary condensation which are widely recognized at adsorption and dehydration isotherm. But in this experiment, the author has employed the values obtained under the same dehydrating process. Although the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ are commonly shown for each and every water-content in the samples, the author has conveniently shown the relation among the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ for the water-activity "a" in the sample.

(B) The analysis of the water-content "g"—water activity "a" curve by the B. E. T. equation.

There are five types as shown in Fig. 11 for the relation between adsorption capacity "v" and equilibrium pressure "p" at the definite temperature which is

generally found in physical adsorption phenomenon[32b].

Fig. 11. General forms of isotherm of physical adsorption.



Unimolecular layer's adsorption equation (6) which was offered by Langmuir[41] in 1918 is fitted to the curve of I-type in Fig. 11, but not fitted to the curves of the other four types:

$$v = abp/(1+ap) \quad \text{-------------------} \quad (6)$$

where "a" and "b" are constants.

However, in 1938 Brunauer, Emmett and Teller[32a] offered an adsorption isotherm, B. E. T. equation fit for the five types in Fig. 11, from dynamic consideration, supposing the multimolecular layer's adsorption. As the detail of the derivation of this B. E. T. equation has been explained by Brunauer et al, Keii[32b], Suga[42a] and Higuch[42b], the author omits details and shows the equation only as follows:

$$p/v\,(p_0 - p) = 1/v_m c + \frac{c-1}{v_m c}\,(p/p_0) \quad \text{----------} \quad (7)$$

where, "p" is equilibrium pressure of the sample, "p_0" is the saturated pressure at the same temperature, "v" is adsorbed amount of water at pressure "p", and "v_m" is adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular layer, and "c" is a B. E. T. constant, this constant depends on the kinds of the sample, but it differs somewhat with the estimating temperatures, and it is supposed to be a kind of index for heat of adsorption. The constant, c, is supposed to be shown as equation (8) from the derivation of equation (7):

$$c = (a_1 g/b_1) \exp E_1 - E_L/RT \quad \text{------------} \quad (8)$$

where, "a_1" and "b_1" are constant, which are concerned in the entrance and the exit of water molecules (that is the condensation and the evaporation of water molecules) at the first layer of the surface of the molecule of the adsorbed substances. "g" is the ratio of "a_i" to "b_i" (a_i/b_i), which is appropriately constant to above the 2nd layer. Here a_i and b_i are constants at 2nd, 3rd, --- ith --- i_∞th layer, which are similarly defined as "a_1" and "b_1" respectively. E_1 is the heat of adsorption at the first layer, and E_L is the heat of liquefaction at the saturated pressure, "p_0". In the derivation of equation (8), it was supposed that the heat of adsorption at the 2nd and higher layers (that is E_2, E_3---E_i---E_∞) are the same as E_1 (E_1 is 9.7 kcal/mol in the case of the adsorption of water molecule). This

is equivalent to saying that the evaporation—condensation properties of the molecules of water at the second and higher layers are the same as those of the liquid state.

Therefore, if it can be supposed that the constants, "a" and "b", are equal at evey layer of $i_1, i_2, --- i_\infty$, the values of "$a_1 g/b_1$" can be represented as 1, then the heat of adsorption $(E_1 - E_L)$ or E_1 will be calculated by the equation (8).

B. E. T. equation (7) as above mentioned was offered to the multimolecular layers adsorption, but if the thickness of the adsorbed layer cannot exceed some definite number, n, the following equation (9) is offered instead of equation (7):

$$v = \frac{v_m c x}{1-x}\left\{\frac{1-(n+1)\, x^n + n x^{n+1}}{1+(c-x)-c x^{n+1}}\right\} \qquad (9)$$

where, "x" is "p/p_o".

In equation (9), if the number of layers, n, is equal to 1 (that is, the adsorption is ended at unimolecular layer), equation (7) is represented as the following equation (10).

$$v = v_m c\,(p/p_o)/\{1 + c\,(p/p_o)\} \qquad (10)$$

This equation (10) is the same as Langmuir's equation (6) which was offered to the unimolecular layer's adsorption.

The explanation above stated is an outline of B. E. T. equation. It is clear from equation (7) that a plot of "$p/v\,(p_o - p)$" against "p/p_o" should give a straight line, whose intercept is "$1/v_m c$" and whose slope is "$(c-1)/v_m c$". From the slope and intercept, the two constants "v_m" and "c" can be evaluated.

The water-content "g"—water-activity "a" curve obtained by the vapour tension method in this experiment is the Ⅱ-type shown in Fig. 11, that is to say a S-type curve. In this case E_1 is larger than E_L, but when E_1 is smaller than E_L, the curve takes the Ⅲ-type as shown in Fig. 11.

Regarding the theory of B. E. T. equation, many discussions have been presented by many investigators, especially Higuchi[42b] has compared this theory of B. E. T. equation with the capillary condensation theory which he himself had asserted; he has pointed out that the B. E. T. theory is not yet complete, and it is open to discussion.

Next, if the amount of water "v_m" necessary to cover the entire surface of the molecule of the sample with unimolecular water layer is determined, the surface area "s" per unit of adsorbed substance will be calculated from the equation (11)[43]:

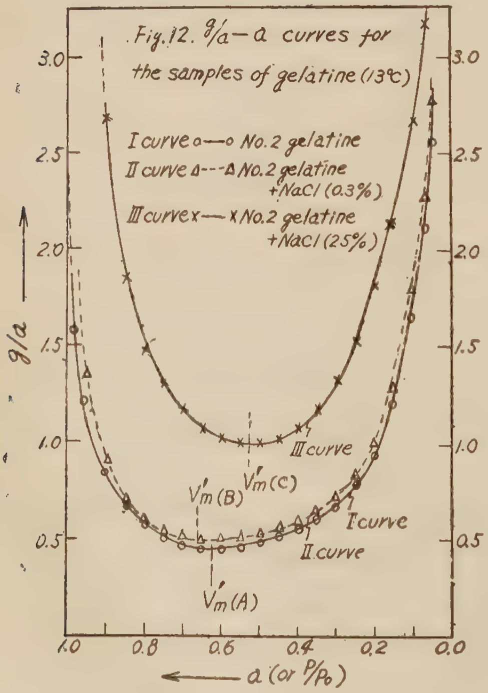
$$s = \frac{v_m\ S_m}{M/N} \qquad (11)$$

where, "N" is Avogadro number, "M" is the molecular weight of the substance

which was adsorbed to the sample (when the substance is water, M is 18), "s_m" is molecular sectional area of the substance which was adsorbed (when the substance is water, "s_m" is 10.45×10^{-16} cm^2.).

When the amount of the adsorbed substance "v" which was obtained from this experiment (in this experiment it is shown as the amount of water per gm of the dried matter of the sample "g"), the equilibrium pressure of water in the sample, p, and the saturated pressure "p_o" of pure water at the same temperature are substituted respectively into the equation (7), and then the relation between "$p/g(p_o-p)$" and "p/p_o" shows as traight line, the values of "v_m" and "c" will be determined. When the values of "v_m" and "c" are substituted into the terms of "v_m" and "c" in the equation (9), and the curve of the relation between the values of "v" (that is "g") which are the amount of the adsorbed substance (when the adsorbed layer "n" is 1, 2, 3 - - - i - - - i_∞) and "p/p_o" (that is "a"), is compared with the observed curve of water-content "g" — water-activity "a", the approximate number of the adsorbed layer, n, will be determined by the resemblance of the curve to the observed curve.

However, in the case of substituting the values of "g" and "p/p_o" (or "a") in equation (7), Briggs[10b] has said that if the estimated value of "p/p_o" (or "a") is below 0.2, the error of the estimation is too large. So if the author plott the value of "gp_o/p" against the value of "p/p_o" according to Bull[31], the curve is a parabola as shown in Fig. 12. The author extrapolated an end of this parabola from 0.2 of the value of "a" to 0.0, and obtained the values of "gp_o/p" corresponding to several points in this curve which shows below 0.2 of the value of "a", and then calculated the values of "g" multiplying them by the values of "p/p_o" (or "a") corresponding to those points. These values of "g" were substituted in equation (7).



(C) The relation between the estimated amount of water

and the calculated amount of water corresponding to every water-activity in the sample to which salts are added.

As stated in Experiment Ⅲ, 1[38a], from the supposition that the difference between the estimated amount of water $g_{2\text{-obs}}$ per gm of the bone dried sample to which salts are added corresponding to a water-activity "a_1" and the estimated amount of water $g_{1\text{-obs}}$ per gm of the bone dried sample to which no salt is added, is the amount of water which hydrates with the added salts showing the same water-activity, the author has obtained in similar calculation with Experiment Ⅲ, 1 in the previous paper, the theoretical curve of water-content "g" — water-activity "a" in the sample, to which salts are added, on the base of the curve of "g—a" in the sample to which no salt is added.

The author compared this theoretical curve obtained by calculation with the curve of "g—a" in the sample to which salts are added. Here, the equivalent weights of salt (m/M, "m" is the quantity of salts added per gm of the dried matter of the sample. "M" is the molecular weight of salt) are as follows:

(a) NaCl was added in proportion of 0.3% to the dried matter of the sample of No. 2 gelatine (at 13° and 8°C)

- - - - - - - - - 0.0513×10^{-3} Mol NaCl

(b) NaCl was added in proportion of 25% to the dried matter of the sample of No. 2 gelatine (at 13° and 8°C)

- - - - - - - - - 4.276×10^{-3} Mol NaCl

(c) $CoCl_2$ was added in proportion of 27% to the dried matter of the sample of No. 2 gelatine (at 8°C)

- - - - - - - - - 1.956×10^{-3} Mol $CoCl_2$

(d) NaCl was added in proportion of 12.77% to the dried matter of Squid meat protein (at 13°C)

- - - - - - - - - 2.183×10^{-3} Mol NaCl

(2) Experimental results and Discussion.

(Ⅰ) Results and Discussion on gelatine.

(A) Experimental results.

The experimental results and the arranged results are shown as in Tables 13 ~17 and in Figs. 13~18.

The sign A in each Table and Fig. indicates the sample of No. 2 gelatine, B the sample of No. 2 gelatine to which NaCl was added in proportion of 0.3% to the dried matter of the sample, C the sample of No. 2 gelatine to which NaCl was

Table 13. Relation between the values of water content "g" and water-activity "a" of the samples of gelatine.

Sample A		Sample B				Sample C				Sample D		Sample E			
(No. 2 gelatine)		(NaCl was added in the proportion of 0.3% per gm of bone dried No.2 gelatine)				(NaCl was added in the proportion of 25% per gm of bone dried No. 2 gelatine)				($CoCl_2$ was added in the proportion of 27% per gm of bone dried No.2 gelatine)		(No. 3 gelatine)			
8°C (p_0 = 0.8045 cm. Hg)		8°C		13°C (p_0 = 1.1231 cm. Hg)		8 C		13°C		8°C		8°C		13°C	
g (gm. of water per gm of bone dried gelatine)	a (or p/p_0) (water-activity of water in the sample)	g	a	g	a	g	a	g	a	g	a	g	a	g	a
2.70	0.979	2.69	0.977	9.87	0.994	2.92	0.916	8.38	0.930	3.23	0.974	4.02	0.987	3.10	0.966
1.63	0.956	0.92	0.850	4.00	0.994	1.38	0.821	4.05	0.922	1.72	0.844	2.71	0.937	1.93	0.950
0.96	0.914	0.60	0.787	1.82	0.983	1.11	0.731	2.26	0.890	1.19	0.731	2.02	0.892	0.98	0.935
0.52	0.792	0.346	0.677	1.24	0.934	0.795	0.602	1.29	0.800	0.86	0.620	1.23	0.908	0.556	0.909
0.333	0.674	0.250	0.438	0.70	0.874	0.646	0.556	0.855	0.681	0.646	0.572	0.829	0.874	0.334	0.789
0.258	0.468	0.181	0.105	0.37	0.724	0.583	0.554	0.738	0.673	0.589	0.530	0.735	0.866	0.258	0.659
0.256	0.415	—	—	0.275	0.548	0.467	0.385	0.644	0.639	0.556	0.495	0.476	0.816	0.205	0.409
0.215	0.324	—	—	0.223	0.330	0.435	0.407	0.533	0.530	0.555	0.495	0.388	0.754	0.158	0.114
0.212	0.260	—	—	0.210	0.181	0.362	0.287	0.403	0.353	—	—	0.223	0.451	—	—
0.192	0.164	—	—	—	—	—	—	0.376	0.223	—	—	0.146	0.128	—	—
—	—	—	—	—	—	—	—	—	—	—	—	0.126	0.074	—	—

Table 14. Relation between the values of water content "g", and of water-activity "a" with the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ of the samples of gelatine at 13°C.

a (or p/p_0)	g					$\Delta\bar{F}$ (kcal/mol)	$\Delta\bar{H}$ (kcal/mol) 8°~13°C					$\Delta\bar{S}$ (cal/mol)				
	A	B	C	D	E	Sample A, B, C, D and E.	A	B	C	D	E	A	B	C	D	E
0.1	0.165	0.180	0.267	0.280	0.140	1.30	12.5	4.10	—	—	1.4	39.2	9.8	—	—	44.5
0.2	0.189	0.201	0.363	0.325	0.178	0.92	7.3	3.35	—	—	8.77	22.3	8.5	—	—	27.4
0.3	0.206	0.219	0.400	0.410	0.199	0.70	7.12	2.73	1.07	—	6.60	22.4	7.1	1.3	—	20.3
0.4	0.221	0.239	0.435	0.465	0.210	0.52	7.12	2.20	2.04	1.8	7.11	23.1	5.9	5.3	4.5	23.0
0.5	0.243	0.268	0.502	0.523	0.219	0.40	6.35	2.04	2.14	3.22	6.35	20.8	5.7	6.1	9.8	20.8
0.6	0.270	0.304	0.613	0.586	0.232	0.30	6.49	2.60	2.66	4.85	5.80	21.6	8.0	8.3	15.9	19.2
0.7	0.330	0.354	0.820	0.700	0.274	0.20	3.12	2.18	2.25	5.45	3.40	10.2	6.9	7.2	18.3	11.2
0.8	0.456	0.472	1.185	0.990	0.360	0.13	1.67	1.77	1.60	4.27	2.70	5.5	5.8	5.1	14.4	9.0
0.9	0.760	0.815	2.42	1.80	0.588	0.06	1.47	1.87	1.01	2.49	2.15	4.9	6.3	3.3	8.5	7.3
0.95	1.150	1.275	—	2.30	1.93	0.03	0.80	1.81	—	—	1.24	2.7	6.2	—	—	4.2

Table 15. Relation between the amount of water (%) and the electrical resistance (50 cycles) of the samples of gelatine. (Estimating temp., 18°C)

Sample B		Sample C				Sample E	
T.W. (Total water content in %)	E. R. (Electric resistance in KΩ)	T. W.	E. R.	T. W.	E. R.	T. W.	E. R.
9.83	2.3	98	0.27	25.7	8.5	64.13	1.55
9.08	0.8	88.7	0.09	22.0	230	59.0	2.10
8.17	0.55	67.5	0.05	21.5	160	55.9	1.95
6.85	0.80	59.5	0.065	21.2	380	52.2	2.70
5.40	1.33	47.5	0.115	21.1	290	47.3	2.80
5.23	1.10	36.9	0.200	20.9	700	41.8	4.80
4.80	2.70	35.2	0.250	20.4	1,200~2,000	37.4	5.5
3.96	9.0	33.0	0.85	20.0	1,000	30.2	15.5
3.61	16.0	31.9	1.3	—	—	18.4	470
3.26	75	30.2	2.2	—	—	14.9	8,000
2.84	300	28.7	2.5	—	—	—	—
2.70	450	27.9	4.8	—	—	—	—
2.30	1,000 over	26.9	5.6	—	—	—	—

Table 16. Analytical results of the "g – a" curves of the sample of gelatine by B. E. T. Method.

Sample	Estimating temp. (°C)	v_m	v_m'	c	E_1-E_L (kcal/mol)	E_1 (kcal/mol)	s (m^2/mg)	v_m'/v_m
A	13°C 8°	0.179 0.186	0.280 0.305	42 38.1	3.78 3.88	13.53	0.645	1.56 1.64
B	13° 8°	0.225 0.230	0.326 0.352	28.1 34.1	3.40 3.54	13.17	0.980	1.45 1.53
C and D	13° or 8°C	0.353	0.530	19.0	3.0	12.7	1.240	1.50
E	13° 8°	0.172 0.196	0.250 0.260	24.5 18.0	3.25 2.90	12.8	0.650	1.45 1.33
Katz(10a)	23°	0.170	0.294	7.85	2.20	11.9	0.600	(1.73)
Bull(31)	25°	0.0873	0.141	17.4	3.04	12.74	0.309	(1.62)

Table 17. Ratio of the theoretical amount of water "$g_{calc.}$" to the observed amount of water "$g_{obs.}$" of the samples of gelatine to which salts were added.

Water activity "a" (13°C or 8°C)	Sample B		Sample C		Sample D
	13°C	8°C	13°C	8°C	8°C
0.1	0.922	0.925	0.903	0.921	0.832
0.2	0.946	0.948	0.815	0.840	0.750
0.3	0.950	0.986	0.855	0.888	0.762
0.4	0.934	0.987	0.915	0.934	0.760
0.5	0.918	0.975	0.944	0.930	0.760
0.6	0.901	0.945	0.946	0.872	0.707
0.7	0.945	0.960	0.942	0.864	0.659
0.8	0.981	0.910	0.980	0.888	0.703
0.9	0.954	—	0.922	—	—
0.95	0.931	—	—	—	—
Average	0.938	0.955	0.904	0.892	0.742

Note: The average value of $g_{cacl.}/g_{obs.}$ of the sample D at 13°C was 0.848 as shown in Table 6 of the previous Exp. Ⅲ, Ⅰ.(38a)

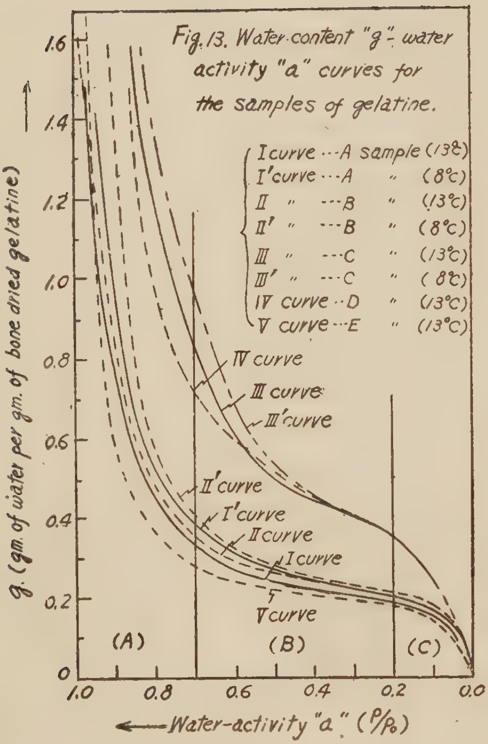


Fig. 13. Water-content "g" - water activity "a" curves for the samples of gelatine.

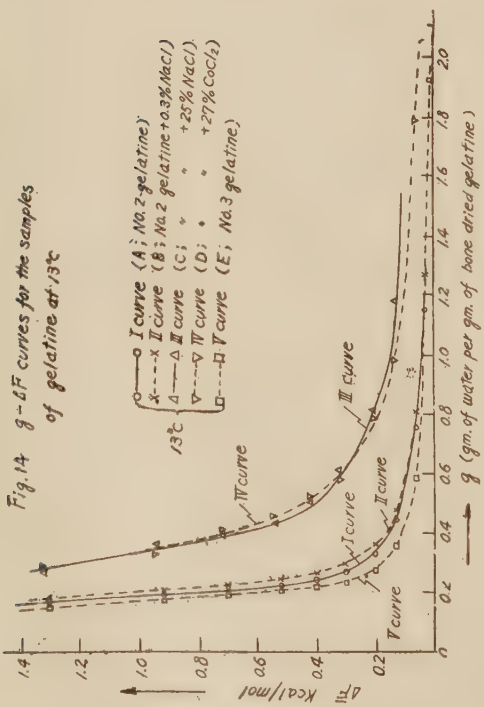


Fig. 14 g - ΔF curves for the samples of gelatine at 13°C

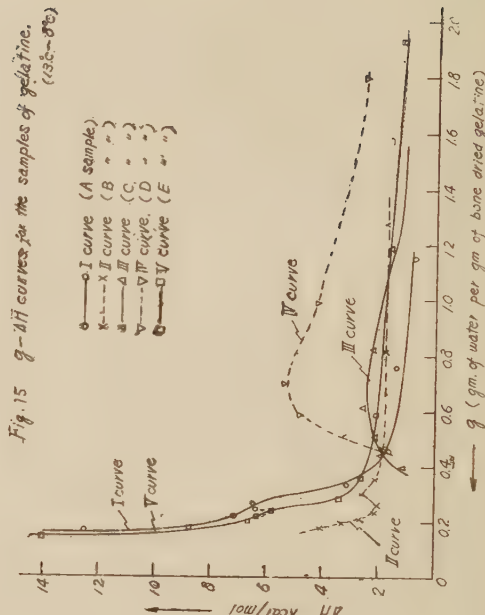


Fig. 15 g - ΔH curves for the samples of gelatine. (13°C ~ 8°C)

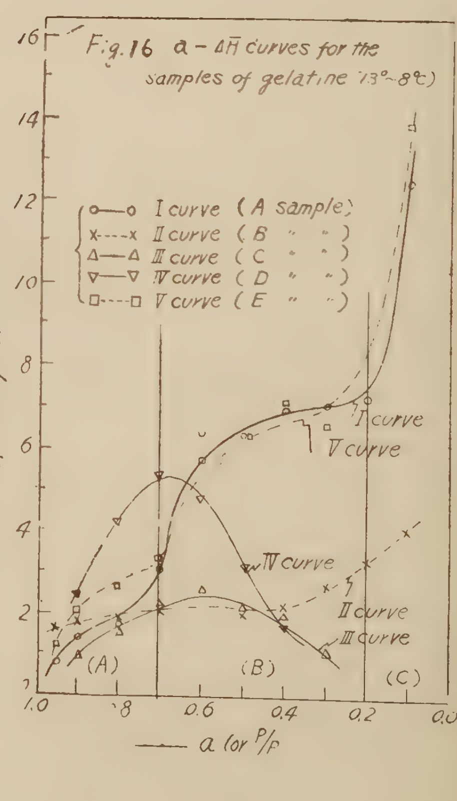
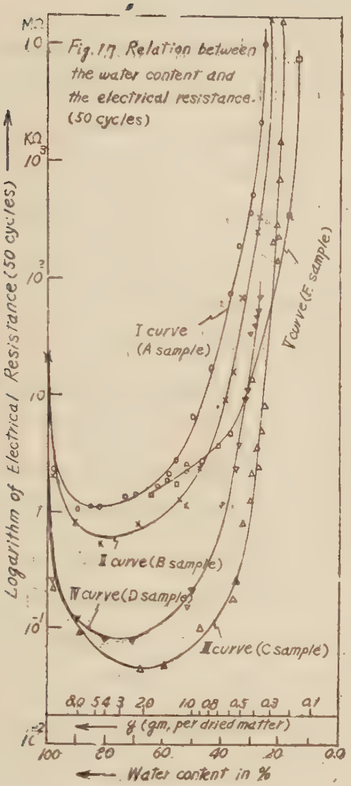
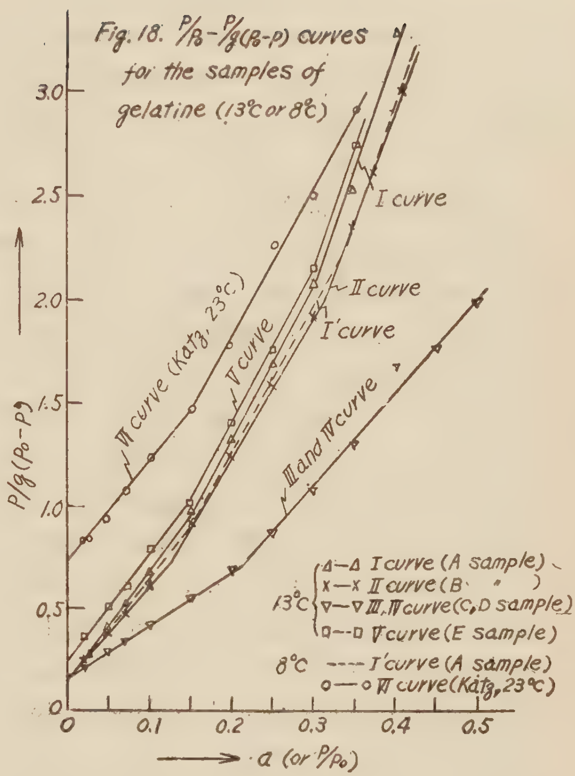


Fig. 16 a - ΔH curves for the samples of gelatine (13° ~ 8°C)





added in proportion of 25% to the dried matter of sample, D indicates the sample of No. 2 gelatine to which cobaltous chloride was added in proportion of 27% to the dried matter of the sample, E the sample of No. 3 gelatine.

Table 13 shows the relation between the water content "g" (the amount of water per gm of the dried matter of the sample of gelatine, gm) and the water-activity "a" in the sample. As the experimental results for the samples of A and D at 13°C have been previously described in the Experiment III, 1, so they are omitted here. The results shown in Table 13 were plotted as in Fig. 13. The curves I, II, III, IV and V in Fig. 13 are the experimental results for the samples of A, B, C, D and E at 13°C respectively, and the curves I′, II′ and III′ are the results for the sample of A, B, C at 8°C respectively. The results for the sample of D, E at 8°C are omitted in Fig. 13.

Table 14 shows the relation among the values of ΔF (13°C), ΔH (13°～8°C) and ΔS (13°C) which were calculated by equations (3), (4), and (5) from the estimated curves of each sample at 13°C and 8°C shown as in Fig. 13, and the water-content "g" of each sample and the water-activity "a" in each sample respectively. The

relation between the water-content "g" and the values of $\Delta\overline{F}$ which are shown in Table 14 is shown as Fig. 14. The relation between the "g" and the value of $\Delta\overline{H}$ is shown as Fig. 15. The relation between the value of $\Delta\overline{H}$ and the water-activity "a" in each sample is shown in Fig. 16, in exchange for "g" shown as in Fig. 15. The relation between the value of $\Delta\overline{S}$ and the water-content "g" is the same as the relation between the value of $\Delta\overline{H}$ and "g", so the Figure to show that relation is omitted here. Table 15 shows the relation between the amount of water in every sample of B, C and E (total amount of water %) and the electrical resistance of 50 cycles(KΩ). The results in Table 15 were plotted as in Fig. 17. In Table 15, the experimental results for the sample of A and D were omitted, because the results for them have previously been shown in Table 4 in the Experiment Ⅲ, 1[38]. The curves Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ in Fig. 17 show the experimental results for each sample of A, B, C, D and E respectively. Table 16 shows the values of B. E. T. constant, c, the amount of the adsorbed water, needed to cover the surface of the gelatine molecule with unimolecular water layer, v_m, the apparent heat of adsorption $E_I - E_L$, the heat of adsosption at the first layer, E_I, and the adsorbed area of surface, s. The value of "v_m'" which is shown in the 4th column of Table 16 is the value of the water-content "g" at the minimum point of the curve of "g/a−g" as shown in Fig. 12. (In Fig. 12, the value of "v_m'" was shown as $v_m'(A)$, $v_m'(B)$ and $v_m'(C)$ for the curves Ⅰ, Ⅱ and Ⅲ for each sample of A, B and C respectively.) The author has added to Table 16, the anlayzed value by B. E. T. equation from the experimental results which were obtained by the vapour tension method by Katz[10a] on gelatine at 23°C, and the values of experimental results obtained by Bull[31] on gelatine at 25°C. Fig. 18 shows the relation between the values of $p/g(p_0-p)$ and p/p_0 of each sample which were obtained from the results shown in Fig. 13. (In the equation of B. E. T. the sign of "g" is shown as "v"). The curves shown in Fig. 18, which show the relation as above stated, are the straight lines in a definite range of the value of "a" (or "p/p_0)". This fact shows that the equation of B. E. T. (7) is certainly applicable to these relations. In Fig. 18, the curve Ⅵ was obtained from the results of Katz. The analyzed results of each sample of B, C, D and E at 8°C were omitted.

Table 17 shows the ratio of the theoretically calculated water content "$g_{calc.}$" as above stated to the observed water content "$g_{obs.}$" at each water activity of each sample of B, C and D to which the salts were added. The result for the sample of D at 13°C was already previously shown in Table 6 in Experiment Ⅲ, 1[38], so the results are omitted from Table 17.

(B) Discussion on the experimental results on gelatine.

From the experimental results obtained, the author will discuss separatlely the following themes: (a) On the samples of gelatine No. 2 and No. 3 to which no salt is added (samples A and E), (b) On the samples of gelatine No. 2 to which salts are added (samples B, C and D).

(a) Discussion on the samples of gelatine No. 2 and No. 3 (samples A and E).

(i) The relation between the water-content "g" and the values of ΔF and ΔH:-

When the "g - a" curves for the same sample which were obtained at 15°C and 8°C were compared with each other, a little difference is seen as shown between the curves I (or II, III) and curve I′ (or II′, III′) in Fig. 13. That is to say, the "g — a" curves which were obtained at lower temperature slipped down to the right hand of the "g — a" curves which were obtained at higher temperature (when we compare the curves at the same water-activity, the curves at lower temperature are situated above the position of the curves at higher temperature).

However, when the water-activity "a" is more than 0.9, the water-content "g" of the curves obtained at higher temperatures is sometimes larger than that of the curves obtained at lower temperatures at the same water-activity.

This fact will be because when the temperature is higher, the thermokinetics of water molecules becomes energetic and the active surface of adsorption becomes newly larger at high moisture.

From that fact, it may be supposed that these thermal phenomena are due to the change of the thermodynamic function.

In the relation between the water-content "g" and the value of $\Delta\bar{F}$ as seen from the curves I and V for each sample of A and E respectively in Table 14, as the water-content "g" is less, the value of ΔF becomes gradually larger. When the value of $\Delta\bar{F}$ shows 0.1～0.2 kcal/mol, and when the "g" is less than 0.3～0.4, the value of ΔF increases rapidly. At the time of decreasing of the amount of water in the sample, the difference, "$\Delta\bar{F}$", between the differential molal free energy "F°" of pure water and the differential molal free energy of water in the sample F, is supposed to be small, while the water in the sample has properties of Free Water. But when the water in the sample loses properties of Free Water, ΔF is supposed to be naturally larger. From those facts, the water, of which the water-content "g" is less than 0.3～0.4, certainly has no property of Free Water.

When the water-content "g" is 0.3～0.4, the electrical resistance of 50 cycles is on the rapid increase as shown in the curves I and V for each sample of A

and E in Fig. 17.

In the relation between the water-content "g" of the samples and the value of $\Delta\bar{H}$ in Fig. 15, with the decreasing of the water-content "g", the value of $\Delta\bar{H}$ increases gradually as shown in the curves I and V. When the water-content "g" is less than 0.3~0.4, the value of $\Delta\bar{H}$ shows 2~3 kcal/mol. And when the water-content "g" is less than 0.3~0.4, the value of $\Delta\bar{H}$ increases suddenly, as well as the change of the value of $\Delta\bar{F}$.

It is a noteworthy fact that when the water-content "g" is in the neighbourhood of 0.2~0.3, a step is recognized in the curve of "$\Delta\bar{H}-g$". This fact was also recognized in the samples of both No. 2 and No. 3 gelatine. From the relation between the water-activity "a" and the value of $\Delta\bar{H}$ which was shown as curves I and V in Fig. 16, the value of $\Delta\bar{H}$ is observed to be a definite value of 3~7 kcal/mol at the part of B of which range of the water-activity "a" is 0.7~0.2. But the value of $\Delta\bar{H}$ is observed to be 0~3 kcal/mol at the part of A of which the range of the water-activity "a" is 0.7~1.0. On the contrary, the value of $\Delta\bar{H}$ is observed to increase suddenly at the part of C, of which the water-activity "a" is less than 0.2, for example the value of $\Delta\bar{H}$ is 12~14 kcal/mol in the neighbourhood of 0.1 of the water-activity "a". Those facts will be discussed by comparing with the analyzed results by B. E. T. equation in the following separate article.

Next, the author will compare the experimental results as above stated with the results obtained by other investigators. Katz[40] has compared the values of ΔF and ΔH in the various high molecular substances, and he has concluded that the values of ΔF and ΔH are almost the same, and there is no change of entropy of that substances at the time of the adsorption of water molecules. But Fricke[41] et al. have made it clear that the entropy of the substances increased with the decreasing of the water-content for the samples of casein and keratin. In the experimental results of the author for the sample of gelatine, it has been recognized that the entropy of the substance (the differential molal entropy), increased with the decreasing of the water-content. Rosenbohm[15] has estimated directly the value of ΔH of gelatine by the ice-calorimetric method, and has said that the limit value of ΔH in the extreme drying is 4 kcal/mol. Rosenbohm's value is not a differential molal enthalpy as the author's results, but 4 kcal/mol of the value of ΔH corresponds nearly to 0.3 of the water-content "g" (23% of the total amount of water). This fact shows that in Rosenbohm's experiment, Bound Water which is strongly combined with the sample perhaps was not completely dehydrated from

the sample, because the sample was dried in room temperature.

According to Bull[31], the value of ΔF is 1.537 kcal/mol, and the value of ΔH is 3.8 kcal (at 25°C) in gelatine. But the values of ΔF and ΔH vary with the change of the water-content, and there was no detailed result concerning this point, so it is difficult to compare these results with each other. However Bull's consideration was concerning the water in gelatine of which the water-activity is less than 0.7, so the value of ΔH of water in the part of B on the "g−a" curve of which the water-activity is 0.7∼0.2 is supposed about 4 kcal. Then the value of ΔH which was 3∼7 kcal/mol in the range of 0.7∼0.2 of "a" in the author's result is nearly in agreement with Bull's result.

(ii) Consideration of analyzed results of the "g−a" curve by B. E. T. equation.

In the relation between the water-activity "a" and "$p/g(p_0-p)$" as shown in Fig. 18, if the water-activity "a" is within the range 0.05∼0.2 (or 0.3), each sample shows a straight line, but if the water-activity "a" is less than 0.05 or is larger than 0.2∼0.3, the angle of inclination of the lines increases rapidly. This fact is recognized as follows: when the molecules of the protein hydrate slowly from the bone dried stage, the hydration follows a path to adsorbing process forming many new active surfaces of adsorption.

The author has shown the curve which was analyzed from Katz's experimental results at 25° by the B. E. T. equation (7) in Fig. 18. Supposing from Table 16 and Fig. 18, as shown in the curves of 25°C, 15°C, one recognizes that there are differences among the values of B. E. T. constant "c", the values of heat of adsorption "E_1-E_L" or "E_1" according to estimating temperatures.

As shown in Table 16, v_m, which is adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular water layer, was average 0.183 gm per gm of the dried matter of No. 2 gelatine, 0.184 gm per gm of dried matter of No. 3 gelatine.

The values of apparent heat of adsorption, "E_1-E_L" of No. 2 gelatine and No. 3 gelatine were 3∼4 kcal/mol, the value of heat of adsorption at the first layer of the adsorption, E_1, was 12∼14 kcal/mol. When those value obtained by the author are compared with the values obtained by Katz and Bull, regarding to the value of "v_m", Katz's value was 0.170 gm per gm of the dried matter of gelatine, which agrees with the author's result; but Bull's value was 0.0873 gm per gm of the dried matter of gelatine, which corresponds to a half of the values of Katz and the author. Regarding Bull's result, the present author can not enter into detailed discussion only from his paper. Regarding values of "E_1-E_L" the results

obtained by Bull and by the author are almost the same.

The values of "v_m'" which are recorded in the 4th column of Table 16 are the values of the water-content "g" which are corresponding to the minimum value of "g/a" for the curve of "g/a—a" in Fig. 12. The minimum points of the curves "g/a—a" for all the samples are situated in the range of 0.6~0.7 of "a". The ratio of "v_m'" to "v_m" is about 1.5 on the average.

According to Bull, at the time of the adsorption of water molecules by the protein molecules, the amount of water needed to form a layer of one molecule of water as the first step of the adsorption to the hydrophilic surfaces which are linked cohering with each other in the molecule of the protein, is shown as v_m. At that time, one molecule of water links with those hydrophilic surfaces with hydrogen bonds. With the advance of hydration, as the second step, while the amount of water which corresponds to "v_m'" (it is about twice "v_m") is adsorbed, it is believed that the two molecules of water enter between the hydrophilic surfaces which are situated among the molecules of protein, and are linked with the protein forming so-called water-water bridge.

According to Bull's results, the ratio of "v_m'" which is obtained from the minimum point of the "g/a—a" curve, to "v_m" which is obtained from B. E. T. equation is 1.62 for the sample of gelatine. The author has also obtained about 1.5 on the average for the gelatine, so this value agrees with those of Bull and Katz.

Katz[29] and Sponsler[36] have recognized from the result of diffraction by x-ray on the gelatine, that when 0.18 gm of water per gm of the dried matter of gelatine is adsorbed, one molecule of water enters among the side chains of the molecules of gelatine and it links with hydrophilic radicals in both the side chains of the molecules of gelatine by hydrogen bonds; and when the hydration advanced and 0.5 gm of water per gm of the dried matter of gelatine is adsorbed, one molecule of water enters among the main chains of the molecule of gelatine, and two molecules of water enter among side chains of the molecules of gelatine; and when the adsorbed amount of water is larger than 0.5 gm, the image of the diffraction by X-ray shows already no clear ring and the arrangement of molecules in gelatine is abnormal. Considering the facts as above stated and the results obtained by the author and by Katz that the value of "v_m" is about 0.17~0.19 as the water-content "g", the value of E_1 is 12~14 kcal/mol, and the energy of one hydrogen bond is about 5 kcal/mol, one molecule of water is supposed to link with both molecules of gelatine by two hydrogen bonds in the part of C in Fig. 13. In the range of 0.17~0.3 gm of the water-content "g" of Fig. 13, that is to say, in the

range from "v_m" to "v_m'" (the part of B whose water-activity is 0.2~0.6 of "a"), two molecules of water are supposed to enter in the molecules of gelatine to form so-called water-water bridge, having one hydrogen bond (This is supposed from the value of "E_1-E_L" is 3~4 kcal/mol.) This conclusion is also obtained from fact that the the fact that the change of the value of differential molal enthalpy, $\Delta\bar{H}$, is 3~7 kcal/mol in the range of 0.2~0.7 of "a".) It is reasonable that water which is situated in the part of B and C of the "g - a" curve which was obtained by the vapour tension method [the value of "a" (or "p/p_0") is less than 0.7] is so-called molecular theoretical Bound Water from the idea of hydrogen binding.

The adsorbed area per unit of gelatine "s" is 0.6~0.65 m^2/mg as shown in Table 16 by the author and by Katz, and about 0.3 m^2/mg by Bull. The difference of these values and reasonableness of these values are reserved for further studies.

(b) Discussion on the sample of gelatine to which salts were added.

(i) Relation of the water-content "g" to $\Delta\bar{F}$ and $\Delta\bar{H}$.

It is clear, from the 1st to 7th column of Table 14, and the curves II, III and IV in Fig 14, that the value of $\Delta\bar{F}$ increases with decreasing of the water-content "g" in both cases of the samples of gelatine to which salts are added or not added.

For example, when the water-content "g" of sample B (to which 0.3% NaCl was added) is 0.3~0.4, the value of $\Delta\bar{F}$ shows 0.1~0.2 kcal/mol, and when the water-content "g" of sample C (to which 25% NaCl was added) and the sample D (to which 27% $CoCl_2$ was added) is 0.6~0.8, the value of $\Delta\bar{F}$ is 0.2~0.4 kcal/mol, and when the water-content "g" is less than 0.3~0.4 or 0.6~0.8 respectively, the value of $\Delta\bar{F}$ increases rapidly.

In comparing the values of $\Delta\bar{F}$ with the same value of "g", the larger the amount of the added NaCl is, the greater the value of $\Delta\bar{F}$ becomes for the samples of B and C. This is due to the fact that a part of water which was present initially in the samples of gelatine hydrates with the salts (NaCl or $CoCl_2$) which were added.

In the relation between the value of $\Delta\bar{H}$ and the water-content "g" of Table 14 and the curves II, III and IV in Fig. 15, it is observed that the values of $\Delta\bar{H}$ increase gradually with initial decreasing of the water-content "g" and afterwards the value of $\Delta\bar{H}$ decreases for the samples of C and D. For example, when the water-content "g" is 0.6~0.8 (total amount of water is about 32~44%), the value of $\Delta\bar{H}$ shows temporarily the maximum value, and then the value of $\Delta\bar{H}$ decreases with the decreasing of the water-content "g", and when the water-content "g" is

0.3~0.4 (the total amount of water is about 23~28%), the value of $\Delta\bar{H}$ shows negative values. The maximum value of $\Delta\bar{H}$ is about 2.5 kcal/mol for the sample of C and is about 5.4 kcal/mol for the sample of D. The decreasing of the water-content "g" below the value of "g" which corresponds to the maximum value of ΔH, is due to the heat of combination of NaCl with water. That is to say, as the heat of dissolution and the heat of dilution of NaCl in water are minus, the NaCl adsorbs the heat which was given out from the gelatine. Therefore the value of $\Delta\bar{H}$ of the sample to which NaCl was added is less than that of the sample to which no NaCl was added.

When the value of $\Delta\bar{H}$ decreases further with decreasing of the water-content "g", the value of ΔH becomes minus, and the sample to which NaCl was added shows the endothermic reaction. When the water-content "g" of gelatine is in the neighbourhood of 0.3~0.4, the gelatine to which NaCl was added attains already a solid state, and the electrical resistance of the gelatine is on the way to rapid increase with the decreasing of the water-content "g", as shown in Fig. 17. Therefore the dehydrating ability of NaCl for water in gelatine becomes stronger in the neighbourhood of 0.3~0.4 of "g", and NaCl perhaps scrambles for water which is hydrated with gelatine. The value of $\Delta\bar{H}$ of B sample of No. 2 gelatine to which NaCl was added in proportion of 0.3% to the dried matter of the sample, as shown in curve II in Fig. 15, is larger than the value of ΔH of curve I of A sample of No. 2 gelatine to which no NaCl was added, when these samples are compared at the same water-content "g". This is perhaps because a part of water in gelatine hydrates with NaCl, and the amount of the water to be bound with gelatine decreases, therefore the water-content "g" decreases and the value of ΔH increases relatively.

Similar conclusion is based on curve IV of the sample of No. 2 gelatine to which $CoCl_2$ was added. That is to say, the value of ΔH of the sample to which $CoCl_2$ was added is larger than the value of ΔH at the same water-content of the sample to which no $CoCl_2$ was added, even after the addition of $CoCl_2$. It was not clear for the sample of No. 2 gelatine to which NaCl was added in the proportion of 25%, whether the same conclusion can be given.

From Fig. 16 which was shown as the relation between the water-activity "a" and the value of ΔH changing from the relation between the water-content "g" and the value of ΔH, the value of ΔH is 2~3 kcal/mol for the sample of B at the part B shown in Fig. 13, that is to say, the water-activity is less than 0.7. This value of ΔH is less than the value of ΔH for the sample of A (curve I) to

which no NaCl was added and for the sample of E (No. 3 gelatine) (curve V). This result is in agreement with Lloyd's[(c)] opinion that the presence of electrolyte such as NaCl in the protein weakens the electrostatic attraction among the side chains of the molecules of the protein.

(ii) Discussion on the analyzed result of the curve of "g—a" by B. E. T. method.

As shown on the curves II, III and IV in Fig. 18, when NaCl was added to No. 2 gelatine in the proportion of 0.3% (in the case of sample B) and 25% (sample C), or $CoCl_2$ was added in the proportion of 27% (sample D), the relation between "$p/g(p_0-p)$" and "p/p_0" shows the linear function when the range of the water-activity "a" is 0.05—0.1 (or —0.21) as well as the samples to which no salt was added. But the greater the amount of added salts, the smaller the angle of inclination of the line is. Therefore the curves II, III and IV are located under curve I which shows for the sample to which no salt was added. Therefore the value of B. E. T. constant "c" which was calculated from the B. E. T. equation from the relation of lines, becomes smaller as shown in Table 16, and each value of the heat of adsorption of "E_1-E_L" and E_1 becomes smaller.

On the other hand, the larger the amount of the added salts is, the greater the value of "v_m" becomes, e g. the value of "v_m" was 0.18—0.19 for the sample of No. 2 gelatine (A) to which no salt was added; it was 0.22—0.23 for the sample (B) to which NaCl was added and it was about 0.35 for the sample of C, D, to which NaCl or $CoCl_2$ were added in the proportion of 25% or 27% to the dried matters of the samples respectively. The value of "v_m" perhaps includes the amount of water hydrated with salts. The amounts of water hydrated with the added salts were calculated from equations (10) and (11), supposing that all the added salts are dissociating (in 100%) in the samples: 0.0018 gm for the sample of B, 0.07—0.1 gm for the samples of C and D. Even if the amounts of water hydrated with added salts are subtracted from the values of "v_m", the remainders are larger than the values of "v_m" of the samples to which no salt is added, perhaps due to the abnormal decreasing of vapour tension by the addition of salts, that is to say due to the abnormal decreasing of water-activity "a". However, the increasing of the value of "v_m" is quite probably due to the crystallisation of a part of added salt for the reason that all the amount of the added salts was not dissolved by water in the gelatine, but a part of the added salts are present in a solid state, and there is the layer of water molecule on the surfsce of solid salts as discussed in Experiment III. 1[(38a)] Such a crystallization out of the solid of salts appears

in clear, when large influence of salts is exerted on Bound Water of which the water-content "g" is less than 0.5, (that is to say, the water-activity "a" is less than 0.7).

Considering a system of the gelatine molecule, salt molecule and water molecule, the number of layer of adsorption "n", or adsorbed area per unit amount of the sample, "s", perhaps increases. These facts are clearly shown in the value of "s" in Table 16 or the value of "n". On the value of the number of layer of adsorption "n" will be discussed later.

(iii) Discussion on the relation between the observed value of the water-content "$g_{obs.}$" and the calculated value of the water-content "$g_{calc.}$".

As shown in Table 17, the ratio of the water content calculated "$g_{calc.}$" to the water-content observed "$g_{obs.}$" at every water-activity of the samples B and C which are No. 2 gelatine to which NaCl was added in the proportion of 0.3 and 25% to the dried matter of the samples respectively, and of the sample D which is No. 2 gelatine to which $CoCl_2$ was added in the proportion of 27% to the dried matter of the sample, is 0.946 both at 13° and 8°C for the sample to which smaller quantities of salt were added such as sample B and it is 0.898 for the sample to which larger quantities of salt were added such as sample C. That is to say, the ratio decreases with the increasing of the amount of adding salt.

The ratio of "$g_{calc.}/g_{obs.}$" for the sample D to which $CoCl_2$ was added is 0.742 at 8°C, 0.848 at 13°C, and 0.795 on the average. This ratio is less than the ratio of the sample C.

As the values of the ratio of "$g_{calc.}/g_{obs.}$" are less than 1, if these values are considered on the curve of "$g-a$" as shown in Fig. 13, the curve of "$g_{calc.}-a$" situates under the curve of "$g_{obs.}-a$" at the same water-activity "a", because the "$g_{obs.}$" is larger than "$g_{calc.}$" (The curve of "$g_{calc.}-a$" is located to the left of the curve of "$g_{obs.}-a$" at the same water-content). Therefore, for three kinds of samples, even if all the added salts are dissociated (to 100%), the abnormal depression of vapour tension, that is to say the abnormal depression of the water-activity naturally still happens. But, as in Rudorff's[34] results discussed in the previous Experiment III, 1[33a], the electrolyte solution always shows such an abnormal depression of the vapour tension. Therefore it is unreasonable to calculate theoretically the value of "$g_{calc.}$" from equations (10) and (11), because this value of "$g_{calc.}$" is widely different from the practical value of water-content "g". The abnormal depression is rightly due to the presence of the electolyte. Therefore, from the fact that the curve of "$g_{obs.}-a$" is situated above the curve of "$g_{calc.}-a$", such

conclusion that water regarding Bound Water (corresponding to water of the difference of "$g_{obs.}$" and "$g_{calc.}$") increase by the addition of salts, does not follow. As discussed in the previous Experiment of Ⅲ, 1, the curve of "$g_{obs.}-a$" is considered to be situated under the practical curve of "$g-a$" at the same water-activity, since the greater the quantities of added salts (NaCl or $CoCl_2$) is, the stronger the force of taking water in gelatine will be.

However, the entire amount of added salts is not considered to hydrate with water in gelatine, but a part of the salt will remain as solid. The theoretical base on which the above conclusion was based, will be stated later on the problem of the number of adsorption "n".

(Ⅱ) Results and Discussion on the Fish meat protein and Fish meat.

(A) Experimental results.

Tables 18～20 and Figs. 19～22 show the experimental results on the Cod (*Gadus macrocephalus*) meat protein (this protein consists mainly of 0 2% NaOH soluble protein that is myotelin.), the common Squid (*Ommastrephes sloani pacificus*) meat protein (this protein consists mainly of 0.8% NaCl soluble protein that is myosin) and fresh raw Atka Mackerel (*Pleurogrammus azonas*) meat.

Table 18 and Fig. 19 show the relation between the water-content "g" and water-activity "a" for every sample, which was obtained by the vapour tension method at 15°C and 10°C.

The curves Ⅰ (or Ⅱ) and Ⅰ′ (or Ⅱ′) in Fig. 19 show the experimental results on the Cod meat protein (or Squid meat protein) at 15° and 10°C respectively. The curves Ⅲ and Ⅲ′ show the results on the Atka Mackerel meat at 15° and 10°C respectively. The curve Ⅱ″ shows the water-content "g" — water activity "a" for the Squid meat protein to which NaCl was added in the proportion of 12 77% of the dried matter of the protein. The curve Ⅱ‴ shows the water-content "g" — water activity "a" which was theoretically obtained for the same sample containing salts after the calculation of the amount of hydrated water from every salt solution at each water-activity as a basis on curve Ⅰ which was obtained at 15°C for samples to which no NaCl was added.

Table 19 shows the relation among the water-content "g", water-activity "a", and the values of ΔF and ΔH. Fig 20 shows the relation between the water-content "g" and the value of ΔH. Fig. 21 shows the linear function between "$p/g(p_0-p)$" ("g" is the same as "v" in B. E. T. equation) and "p/p_0" (or "a") which was obtained from the curves of water-content — water-activity for every sample which

Table 18. Relation between the values of water content "g" and the water-activity "a" of Cod fish meat protein, a Squid meat protein and the raw Atka Mackerel meat at 15° or 10°C.

Sample A※				Sample B※		C※		Sample D※				Sample E※	
15°C(p_0=1.2788cm.Hg)		10°C(p_0=0.9209cm.Hg)		15°C		15°C		15°C		10°C		10°C	
g_1	a_1	g_2	a_2	$g_{3\,(obs.)}$	a_3	$g_3'_{(calc.)}$	g_3'/g_3	g_4	a_4	g_5	a_5	g_6	a_6
4.99	0.968	6.1	0.947	5.6	0.948	—	—	2.90	0.971	6.14	0.947	2.08	0.998
1.20	0.864	2.2	0.953	1.00	0.748	—	—	1.185	0.919	2.20	0.953	1.18	0.914
0.75	0.850	0.419	0.874	0.435	0.679	0.436	1.001	0.751	0.879	0.42	0.874	0.725	0.824
0.402	0.851	0.215	0.683	0.253	0.543	0.310	1.225	0.325	0.804	0.215	0.683	0.413	0.668
0.251	0.740	0.146	0.325	0.184	0.327	0.219	1.190	0.133	0.405	0.146	0.325	0.246	0.548
0.135	0.299	0 108	0.123	0.166	0.246	0.194	1 168	0.104	0.187	0.108	0.081	0.198	0.436
0.095	0 065	0.097	0.075	0.148	0.195	0.180	1.217	—	—	0.098	0.081	0.169	0.319
—	—	—	—	0.147	0.113	0.146	0.986	--	–	–	—	0.161	0.222

Note: (1) The average value of $g_{calc.}/g_{obs.}$ of Squid fish meat protein was 1.153 in the range of 0.7～0 05 of "a".
(2) The estimating curve for the Atka Mackerel meat at 15°C shown as Ⅲ curve in Fig. 19.

Table 19. Relation between the values of water content "g" and of water-activity "a" with the values of ΔF and ΔH of the samples of Cod fish meat protein and the raw Atka Mackerel meat at 15°C.

a (or p/p_0)	g		$\overline{\Delta F}$ (kcal/mol)	ΔH (kcal/mol) 10°～15°C	
	Sample D※	Sample E※	Sample D and E	Sample D	Sample E
0.1	0.078	0.097	1.25	12.9	—
0.2	0.107	0.124	0.87	13.9	—
0.3	0.120	0.140	0 65	16.5	16.9
0.4	0.135	0.157	0.50	13.4	16 5
0.5	0.151	0.183	0.38	8.00	10.6
0.6	0.179	0.227	0.28	2.98	5.96
0.7	0.230	0.304	0.19	0 45	4.86
0.8	0.325	0.480	0.12	—	3.14
0.9	0.900	1.220	0.07	—	—

Table 20. Analytical results of "g－a" curves of the samples of fish meat protein and the raw fish meat by B.E.T. method.

Samples※	Temp.	v_m	v_m'	c	$E_l - E_L$ (kcal/mol)	E_l (kcal/mol)	s (m^2/mg)	v_m'/v_m
D	15°C	0.136	0.173	11.1	1.39	11.16	0.478	1.27
A and D	{A…10°C D…15° or 10°C	0.111	0.192	30.3	1.91～1.95	11.61～11.65	0.391	1.73
E	15°C	0.111	0.183	30.3	1.95	11.65	0.391	1.65
	10°C	0.146	0.228	16.8	1.59	11.29	0.514	1.56
B	15°C	0.164	0.220	17.33	1.63	11.33		1.34

※in Tables 18～20: Sample A…Squid fish meat protein (0.8% NaCl soln. soluble), Sample B…Squid fish meat protein to which NaCl was added in the proportion of 12.77% per gm of bone dried sample, C…Rotio of "$g_{calc.}$" to "$g_{obs.}$" of Squid fish meat protein at each water-activity, Sample D…Cod fish meat protein (0.2% NaOH soln. soluble), E…Raw Atka Mackerel meat.

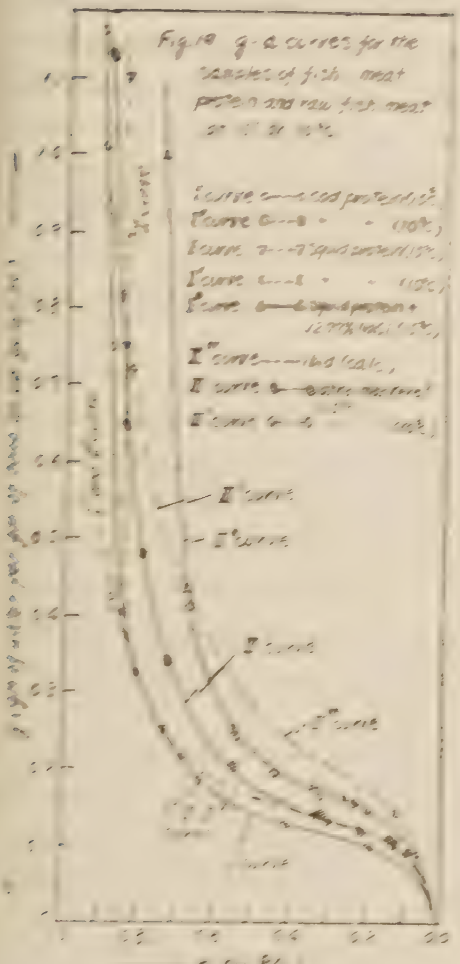


Fig.19 q-a curves for the samples of fish meat protein and raw fish meat

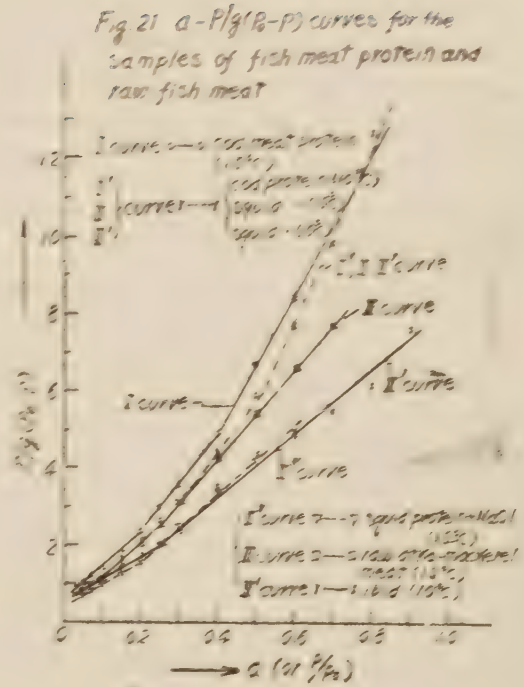


Fig.21 a-P/q(P0-P) curves for the samples of fish meat protein and raw fish meat

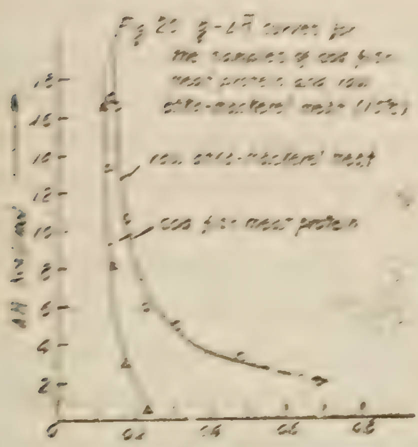


Fig.20 q-ΔH curves for the samples of cod fish meat protein and raw atka-mackerel meat (15°C)

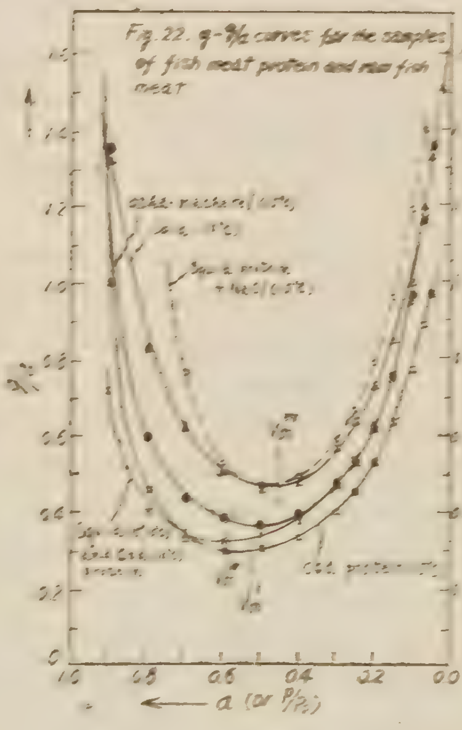


Fig.22. q-Q/a curves for the samples of fish meat protein and raw fish meat

is shown in Fig. 19. Table 20 shows the values of the adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular water layer, v_m, B. E. T. constant "c", heat of adsorption at first layer, E_1, apparent heat of adsorption, E_1-E_L, at indefinite adsorption, and adsorbed area per unit of adsorbed substance, s. "v_m'" which is shown in the 4th column of Table 20 is the value of the water-content "g" at the point of curvature of the curve of "g/a—a" which is shown in Fig. 22.

(B) Discussion on the experimental results obtained on the fish meat protein and raw fish meat.

In Fig. 19, curve I′ shows the results at 10°C on the Cod meat protein and curves II and II′ show the results at 15° and 10°C on the common Squid meat protein respectively. These curves I, II and II′ overlap one another when the water-activity "a" is below 0.8. When the water-activity "a" is larger than 0.8, the water-content "g" becomes larger at the same water-activity at higher temperatures. This fact was also recognized in the case of the sample of gelatine; this is perhaps due to the increasing of the area of the active surface of adsorption according to the increasing thermokinetics of water molecules at higher temperatures.

When curve III which shows the results on the fresh raw Atka Mackerel meat is compared with curves I and II which were obtained on the protein of each fish meat, the water-activity "a" of the sample of only fish meat is less than in the samples of fish meat protein at the same water-content according to the existence of salts in the sample.

(a) Discussion on the relation between the water-content "g" and the value of ΔH.

In the relation between the water-content "g" and the values of ΔH for Cod meat protein and raw Atka Mackerel meat which is shown in Table 19 and Fig. 20, the value of ΔH increased gradually with the decreasing of the water-content "g" as well as for gelatine. For example, for the raw Atka Mackerel fish meat, the value of ΔH is 4~5 kcal/mol at the neighbourhood of 0.3 of the water-content "g" (about 23% of the amount of water in the sample), and is 16 kcal/mol at the neighbourhood of 0.15 of "g" (about 13% of the amount of water); for the Cod meat protein, as well as for the raw Atka Mackerel meat, the value of ΔH is less than 0.2~0.5 kcal/mol at the neighbourhood of 0.2 of "g" (about 20% of the amount of water), and the value of ΔH increased rapidly with the decreasing of the water-content "g". The water-activity "a" corresponding to the water-content "g" at the rapid increasing of the value of ΔH is about 0.7 for the raw

Atka Mackerel meat, and is about 0.74 for the Cod meat protein according to Fig. 19. The value of ΔH increases rapidly when the water-activity "a" is less than these values. The properties of water in the parts of B and C of which the water-activity "a" is less than 0.7, are different from the properties of water in the part of A of which the water-activity "a" is above 0.7, that is to say, the former perhaps combines with the molecules of the fish meat protein by the hydrogen bonds. This conclusion holds as in the case of gelatine.

(b) Discussion on the analyzed result of the curve of "g—a" by B. E. T. equation.

As shown in Fig. 21, the relation between the water-activity "a" (the relative vapour pressure "p/p_o") and "$p/g(p_o-p)$" is a linear function when the water-activity "a" is in the range of 0.05~0.2 in all the samples, but the angle of the inclination becomes larger with several portions by new increasing of the area of the adsorption surface, when the water-activity "a" is more than 0.2 just as in the case of gelatine.

The values of B. E. T. constant "c", the values of "v_m" which are adsorbed amount of water, when the entire adsorbent surface is covered with a complete unimolecular water layer, the values of heat of adsorption at the first layer of the adsorption, E_1, the values of apparent heat of adsorption "E_1-E_L", where the adsorption comes to the state of saturation and the values of the surface area per unit of adsorbed substances, s, were as shown in Table 20. Here, the values of "c" and "v_m" were calculated from the B. E. T. equation (7) after substituting every value of "p/p_o" and "$p/g(p_o-p)$" to the terms of that equation. The values of "E_1-E_L" were calculated from equation (8).

The values of "v_m" is 0.10~0.14 for the Cod meat protein and the common Squid meat protein, and range 0.1~0.15 for the raw Atka Mackerel meat. The values of "v_m" of gelatine (0.18~0.19) are larger than the "v_m" of the fish meat protein. The values of "s" are 0.4~0.5 for all the samples, those values are the same for the gelatine molecule. The discussion on the values of "s" is left for future experiment. The values of "v_m'" which are the value of the water-content "g" corresponding to the minimum value of "g/a" for the curve of "g/a—a" for fish meat protein and raw fish meat in Fig. 22, are shown in Table 20. The ratio of "v_m'" to "v_m" is 1.51 on the average, almost the same as for gelatine. The values of "a" corresponding to every "v_m'" is in the range of 0.5~0.7.

From those facts, water in the fish meat protein and raw fish meat at the parts of B and C of which the water-activity "a" is less than 0.7 is molecular

theoretical Bound Water on the basis of the idea of hydrogen bond, as well as in the case of gelatine.

(c) Discussion on the protein of the common Squid meat to which salts are added.

The relation between "$p/g(p_o-p)$" and "p/p_o" shows linear form (shown as II″ curve in Fig. 21) when the water-activity "a" is in the range of 0.05～0.2 for the Squid meat protein to which NaCl was added in the proportion of 12.77% to the dried matter of the sample. When the II″ curve was compared with II-curve which pertains to the sample to which no salt was added, the angle of inclination of curve II″ is smaller than that of the II curve. Consequently, the values of "c" which were calculated from the B. E. T. equation (7), E_1-E_L, and "E_1" become samller, on the other hand, the values of "v_m" and "s" become large. These facts were also recognized in the case of the addition of salts to gelatine This is generally clarified that the heat of adsorption decreases by the addition of salts.

The ratio of "$g_{calc.}$" which was calculated from equations (10) and (11) for the Squid meat protein to which salts were added at every water-activity "a", to "$g_{obs.}$" was shown in the 12th column in Table 18. The ratio was 1.153 on the average in the range of water-activity 0.7～0.05. The curve of "$g_{calc.}-a$" is situated above the curve "$g_{obs.}-a$" at the same water-activity "a". (The former is situated to the right of the latter at the same water-content "g").

Therefore, as in Experiment III, 1[35] discussed on the relation between the sample No. 2 gelatine and the salt, a part of the added salt hydrated with water in gelatine, and the rest crystallized out as solid. When to the Squid meat protein NaCl was added, the same result is obtained in the case of gelatine.

(III) Discussion on the number of adsorption layers, "n".

Brunauer et al[32a] have offered to equation of adsorption of definite layer (9) besides equation (7) of adsorption of indefinite layer. If the values of adsorbed water-content "g" and the water-activity "a" which were practically estimated are substitued to the terms of equation (7) and the relation of "$p/g(p_o-p)$" and "p/p_o" is obtained, then the values of "v_m" and B. E. T. constant "c" will be calculated. If these values of "v_m" and "c" are substitued to the terms of equaton (9), the values of the amount of adsorbent "v" (the same as the water-content "g") will be calculated in the case of the numbers of adsorption layers "n" as 1, 2, 3, 4, • • • corresponding to the values of "x" (this is equal to the water-activity "a" or "p/p_o)". In this case, the values of "c" and "v_m" are difinite, and various curves of "g－a"

corresponding to the numbers of adsorption layers of the samples are obtained.

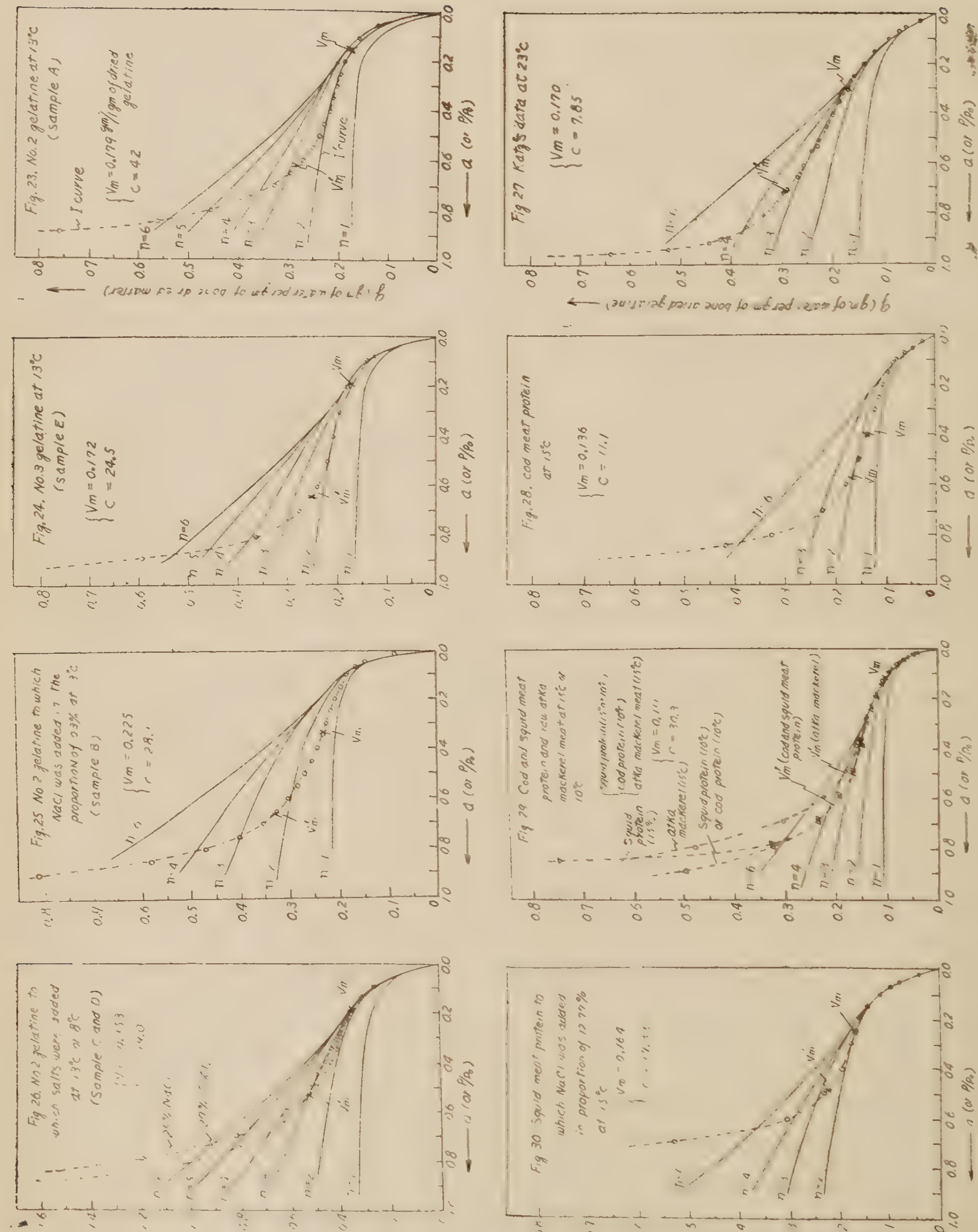
Figs. 23, 24, 25, and 26 show the curves of "g—a" which were plotted for the samples of No. 2 gelatine, No 3 gelatine, No. 2 gelatine to which NaCl was added in the proportion of 0.3% to the dried matter, No. 2 gelatine to which NaCl or $CoCl_2$ was added in the previous proportions of the dried matter, respectively, in the case of various numbers of adsorption layers "n" (n=1~6) at 13°C. Fig. 27. shows the curves of "g—a" which were obtained for the numbers of the adsorption layer "n" (n=1~6) according to equation (9) on the basis of the values of "v_m" and "c" of Katz at 23°C. The values of "v_m" and "c" of those samples were used from Table 16. The dotted lines in those Figures show the curves of "g—a" which were observed for every sample.

Fig. 28, 29, and 30 show the curves of "g—a" which were plotted for the samples of Cod meat protein, common Squid meat protein, raw Atka Mackerel meat and the common Squid meat protein to which NaCl was added in the proportion of 12.77% to the dried matter, respectively, in the case of various numbers of adsorption layers "n" (n=1~6) at 15° or 16°C. The dotted lines in those figures show the curves of "g—a" which were observed for every sample. The values of "v_m" and "c" of those samples were used from Table 20

Brunauer et al. said that equation (7) becomes a very good approximation to equation (9), when the value of "a" is small and the numbers of adsorption layers "n" are as large as 4~5. So the observed curve of "g—a" should become a very good approximation to the curves of "g—a" which are obtained by the B. E. T. equation (9).

However, as in Fig. 23 which shows the curves for the sample of No. 2 gelatine, the observed curve of "g—a" (curve I) agreed with the curve of "g—a" (curve I′) which is obtained when the number of adsorbed layers "n" is 2, when the values of the water-activity "a" are in the range of 0.40~0.0, but when the water-activity "a" is more than 0.4, the observed curve I slips rapidly away to one side of curve I′. And when the water-activity "a" is more than 0.85, the observed curve I keeps away in its upper part from the curve of "g—a" at which the number of adsorption layers "n" is 6. These facts are perhaps due to the increasing of numbers of adsorption layers by yielding many new surfaces of adsorption with the increasing of the' amount of adsorbed water "g" in the process of the adsorption.

Table 21 shows the values of "n" which are adequate to the initial process of adsorption from the relation between the curves I and I′ as shown in Figs.



23—30, and the values of "a" corresponding to the values of "n" in the applicable range of the curves of I and I′.

Table 21. Relation between the number of absorption layers "n" and the water-activity "a" corresponding to the values of "x" in the applicable range of the curves I and II in Figs. 20~25.

Samples	No.1 gelatin		No.2 gelatin		No.1 gelatin to which NaCl [illegible] was added		No.1 gelatin to which NaCl 12% or 25% was added	[illegible]
Temp.	15°	25°	15°	25°	15°	25°	15° and 25°	25°
Number of absorption layers "n"	2	2~3	2	2	1~2	1~2	3	3
Applicable range of water-activity "a"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 21. (Continued)

Samples	[illegible] wool protein		[illegible] wool protein	[illegible] wool protein to which NaCl [illegible] was added	[illegible]	
Temp.	15°	15°	15° and 15°	15°	15°	15°
Number of absorption layers "n"	2	3	2	2~3	3~4	3
Applicable range of water-activity "a"	[illegible]~0.55	[illegible]~0.5	[illegible]~0.5	[illegible]~0.55 ("x" was [illegible] in the range of [illegible])	[illegible]~0.55	[illegible]~0.85

According to Figs 20~25 and Table 21, the value of "n" in the initial process of absorption was 2 or 3 and the applicable range of the values "a" in the observed curve is 0.1~0.55 or 0.6.

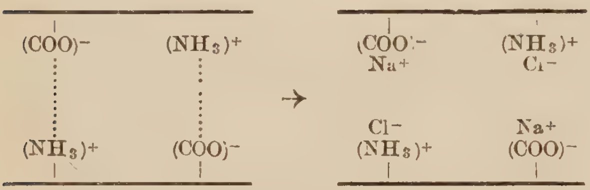
The author can explain that the value of "n" is 2 under the same assumption as Bull[illegible]. That is to say, protein molecules in the solid state are linked together to form coherent planes whose opposed surfaces are hydrophilic. Water is adsorbed between these planes. Therefore the number of absorption layers is naturally 2. With the advance of hydration, when the so-called water-water bridge was formed, the value of "n" is perhaps 3. With the further advance of hydration, the protein tends to dissolve in water and proceeds to indefinite absorption. The manner of binding of hydrophilic plane with water molecule are different with the kinds of protein, e.g., with the difference of fibre protein such as keratin, fibroin or sphero-protein such as ovalbumin, [illegible], [illegible] and others. The fibre protein has chain-bindings which linked together in parallel and the sphero-protein has some cyclo-bindings in a part of its structure.

However, according to Table 21 obtained by the author, various sphero-proteins used will also have chain-binding in the parallel state with hydrophilic plane.

Considering from the influence of the addition of salts, according to Table 21, Figs 22 and 24, the number of absorption layers "n" is 2 or 3 except in case of the addition of salts in small proportion to the dried matter; e.g., the sample to

which NaCl was added in the proportion of 0.3%. In general, by addition of salts, "n" will increase by one.

In comparing with the results of the common Squid meat protein, the same sample to which salts were added and the raw Atka Mackerel meat which were shown in Figs. 29 and 30 respectively, "n" of the sample to which salts were already added in large quantity is recognized to be 3 from the initial process of adsorption. The fact that "n" is increased by one by means of the addition of salts is perhaps because in the protein to which salts were added, the salts link with hydrophilic radical in the side chain of the protein as follows:



This state of binding was offered by Lloyd[4b]. Therefore, three of the adsorption layers were formed in the chain-bindings of the protein, as well as the linkage of two molecules of water in the chain-bindings of the protein. Consequently the values of "v_m" and "s" should naturally increase.

As shown in Figs. 23~30, it is commonly recognized that the adsorption process follows rapidly to the adsorption of indefinite layers more than the water-content "g" corresponding to the point of "v_m'" in figures. The values of the water-activity "a" corresponding to the values of "v_m'" is less than 0.6~0.7 (in the part of B). This is due to the fact that water whose water-activity "a" is less than 0.7 in the refined sample to which no salt was added, is the so-called molecular theoretical Bound Water.

(3) Conclusion

The author has discussed the thermodynamic properties of water below 0.7 of "a" in the curves of the water-content "g" — water-activity "a" for the common Squid meat protein, Cod meat protein, raw Atka Mackerel meat and gelatine, which were determined by the vapour tension method at two different temperatures, and discussed also the analyzed result by B. E. T. method; the author is certain that water below 0.7 of "a" in the curve of "g — a" should be the so-called molecular theoretical Bound Water on the basis of the idea of hydrogen bond formation.

4. Comparison of vapour tension method with other thermodynamic method.

As in the Experiment III, I[38a] stated, Briggs[10b, c] has said that the amount of Bound Water in the sample varies with the water-activity "a", e. g , "a"

corresponding to the concentration of solute in the solvent method, and "a" corresponding to the temperature to freeze the sample in the freezing method, and he has examined the relation of the water-activity "a" and the water-content "g" (gm of water per gm of the dried matter) by isotenoscope method (vapour tension method) and by the method of estimating the depression of the freezing point using the arabian gum, Na-casein; then he has ascertained that the observed values which were obtained by these methods are present on the same contineuous curve of "g—a". According to Briggs' result, water having string binding were estimated in the range of small value of "a" on the curve, but in the range of large value of "a", water containing weak binding, were also estimated at the same time with these water having strong binding.

Recently Higashi and Nukazawa[1c] have arranged the results which had previously been obtained by Moran[7], Lloyd and Moran[4b] or Katz[10a] by various thermodynamic methods using gelatine, and when the values of water-content "g" are plotted in the ordinate, and the values of water-activity "a" are plotted in the abscissa, the values obtained by the freezing method, the swelling tension method, colorimetric method (except the vapour tension method by Katz) are observed to be present on the same curve of "g—a". But the results obtained by Katz by means of the vapour tension method were observed to be much below the above mentioned curve, when the value of "a" is less than 0.95.

Higashi et al. have said that the results obtained by Katz were estimated at 23°C and the curve of the water-content "g"—water activity "a", obtained by vapour tension method, varies generally more or less with temperatures. Therefore, when the results obtained by Katz are to be compared with results obtained by other methods, the results by vapour tension method should be obtained at 0°C.

Here, the present author will describe his results of comparing of No. 2 geltine and No. 3 gelatine at 13° and 8°C by means of vapour tension method with the curves of "g—a" obtained by various other thermodynamic methods for the sample of gelatine.

(1) Examintion method.

(I) Calculating of the values of the water activity "a" in the sample by every thermodynamic method.

In the case of vapour tension method, the value of "a" is manifested approximately by "p/p_0" in equation (3) written in Exp. II, 3, (1), (iii), (A) which shows the water activity.

In the freezing method, the value of water-activity "a" is manifested by

equation (1) neglecting the heat of dissolution while the values of water-content are large.

With the decreasing of the amount of water-content, (that is to say, with becoming high in the concentration of the sample), the heat of dissolution becomes large, and can not be neglected. If the freezing point of a sample is T′°K, the water-activity "a" at T″°K can be calculated from equations (12) and (13).

$$\log a + 0.004211\,(273.1 - T') + 0.0000022\,(273.1 - T')^2 = x \text{ - - - - - - - - } (12)$$

$$x = -L\frac{T'' - T'}{2.303RT'T''} + (C_p - C_p^\circ)\left(\frac{T'' - T'}{2.303RT'} - \frac{1}{R}\log\frac{T''}{T'}\right) \text{ - - - - - - - - } (13)$$

where L is the difference between the differential heat content of water in the sample and the pure water at T″°K, C_p and C_p° is the heat capacity at constant pressure of water in the sample and the pure water respectively.

If 273.1°K (0°C) is substituted to the term T″°K in equations (12) and (13), the curve of "g−a" at 0°C is calculated from the relation between the water-content "g" and freezing point T′°K.

In the swelling tension method, the relation between the swelling tension of the sample "p" and the water-activity "a" is calculated approximately from equation (14) by Katz.

$$-p = \frac{RT}{v_0}\ln a \text{ - } (14)$$

where "v_0" is molal volume of water in the sample. The greater the value of "p" (the smaller the water-contuet of the sample is), the smaller the value of "a" is. With approaching of the value of "p" to 0, the value of "a" approaches to 1.

As above stated, every relation between "g" and "a" is obtained from various thermodynamic estimating methods of Bound Water.

(Ⅱ) Calculating of the values of the water activity "a" from the author's results by the vapour tension method.

To compare with each other the relations of "g" and "a" obtained by various thermodynamic methods, the relation between "g" and "a" should be compared from the results obtained at 0°C.

However, the author is very sorry to be unable to carry out the vapour tension method at 0° using gelatine or fish meat.

Accordingly the relation of "g−a" was calculated by the equation of Clausius-Clapeyron (written in the Experiment, equation (4), Ⅲ, 3) from the results obtained at 15° and 8°C for the samples of No. 2 gelatine and No. 3 gelatine, and then the author obtained the values of water-content "g_1", and differential molal enthalpy ΔH.

$$\Delta H = -\frac{RT_1T_2}{T_1 - T_2}(\ln a_2/a_1)$$

where "a_2" and "a_1" are the water-activities at $T°_1$ and $T°_2$K corresponding to the definite water content "g_1".

Supposing that the values of ΔH at 0°~13°C or 13°~23°C are constants respectively, the water-activity "a_2" at 0° or 23°C was reckoned from the same water-content "g_1". The results at 23°C were calculated to be compared with the results obtained by Katz.

If the values of ΔH, a_1 and T_1 are known in the above equation, the value of "a_2" can be calculated. But the values of the water-content "g_1" corresponding to the known values of "a_1" varies with the estimating temperatures T_1. If there are two different temperatures, A and B, and if $(T_1)_A > (T_1)_B$, then it becomes $(g_1)_A < (g_1)_B$. That is to say, it is clear that in the case of the same water-activity "a_1," the lower the estimating temperature is, the greater the value of "g_1" becomes, as also in the Experiment Ⅲ, 3. Therefore, the values of "a_2" vary with the values of "g_1" which should be as a standard. Therefore, the values of the water-activity "a_2" of which T_2 is 0°C and 23°C respectively are calculated from the values of the water-content "$(g_1)_{13°C}$" and "$(g_1)_{8°C}$" of which A and B of estimating temperatures are 13°C and 8°C respectively. The interpolate lines at every point which are on the relation between the value of "a_2" and "$(g_1)_{13°C}$" or "$(g_1)_{8°C}$" plotted were regarded as the curve of "g−a" at 0° or 23°C.

The values of "g", "a" and ΔH were employed from Table 14 as given in Experiment Ⅲ, 3.

The curves of "g−a" which were obtained by the method as above stated at 0°C and 23°C by author were compared with the curve of "g−a" which was calculated from equations (12), (13) and (14) by Higashi (Moran, Lloyd and Moran and Katz by means of freezing method, calorimetric method and swelling tension method using gelatine), and with the curves of "g−a" which were obtained by means of vapour tension at 23°C by Katz.

(3) **Results and Consideration.**

The curves of "g−a" at 0° and 23°C which were reckoned from the results obtained at 13° and 8°C for the sample of No. 2 gelatine are shown in Fig. 31 (Table 22). The similar curves for the sample of No. 3 gelatine are shown as in Fig. 32 (Table 23).

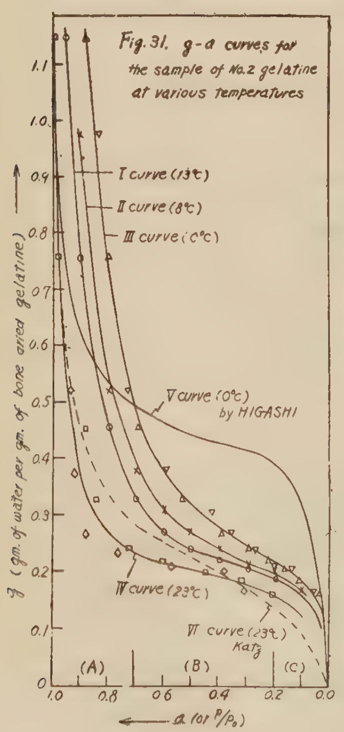
In Figs. 31 and 32, the curves Ⅰ and Ⅱ are the observed curve of "g−a" at 13° and 8°C respectively, the curves Ⅲ and Ⅳ are the calculated curves at 0°

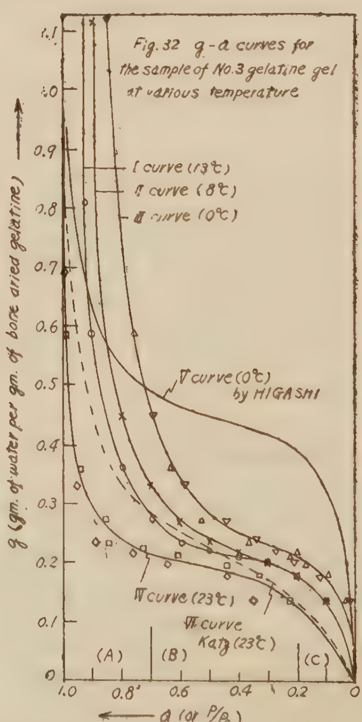
Table 22. Relation between the values of water content "g" and the water-activity "a" of the sample of No. 2 gelatine at 23,° 13°, 8° and 0°C.

Water-activity $(a_1)_{t1=13°C}$ or $(a_2)_{t2=8°C}$	No.2 gelatine Water-content $(g_1)_{t1=13°C}$	$\Delta\bar{H}$(kcal/mol) (13°~8°C)	Water-activity $(a_0)_{t0=0°C}$	Water-activity $(a_3)_{t3=23°C}$	No.2 gelatine Water-content $(g_2)_{t2=8°C}$	Water-activity $(a_4)_{t4=0°C}$	Water-activity $(a_5)_{t5=25°C}$
0.1	0.165	12.5	0.035	0.210	0.170	0.052	0.310
0.2	0.189	7.3	0.108	0.324	0.207	0.136	0.387
0.3	0.206	7.12	0.165	0.458	0.212	0.207	0.572
0.4	0.221	7.12	0.223	0.611	0.240	0.276	0.762
0.5	0.243	6.35	0.294	0.729	0.272	0.358	0.889
0.6	0.270	6.49	0.394	(0.882)	0.310	0.427	(1.08)
0.7	0.330	3.12	0.539	0.843	0.380	0.590	0.929
0.8	0.456	1.67	0.696	0.884	0.524	0.733	0.931
0.9	0.760	1.47	0.796	0.982	0.980	0.833	(1.03)
0.95	1.150	0.8	0.888	0.996	1.40	0.911	(1.02)

Table 23. Relation between the values of water content "g" and the water-activity "a" of the sample of No. 3 gelatine at 23,° 13°, 8° and 0°C.

Water-activity $(a_1)_{t1=13°C}$ or $(a_2)_{t2=8°C}$	No.3 gelatine Water-content $(g_1)_{t1=13°C}$	$\Delta\bar{H}$(kcal/mol) (13°~8°C)	Water-activity $(a_0)_{t0=0°C}$	Water-activity $(a_3)_{t3=23°C}$	No.3 gelatine Water-content $(g_2)_{t2=8°C}$	Water-activity $(a_4)_{t4=0°C}$	Water-activity $(a_5)_{t5=25°C}$
0.1	0.140	1.4	0.031	0.230	0.140	0.021	0.358
0.2	0.178	8.77	0.096	0.331	0.178	0.126	0.443
0.3	0.199	6.60	0.173	0.444	0.202	0.212	0.646
0.4	0.210	7.11	0.220	0.611	0.220	0.276	0.762
0.5	0.219	6.35	0.204	0.729	0.237	0.358	0.889
0.6	0.232	5.80	0.369	0.847	0.270	0.443	(1.01)
0.7	0.274	3.40	0.527	0.856	0.333	0.586	0.953
0.8	0.360	2.70	0.638	0.939	0.450	0.695	(1.02)
0.9	0.588	2.15	0.752	(1.02)	(1.120)	0.848	(1.09)
0.95	1.93	1.24	0.856	(1.02)	—	—	(1.06)





and 25°C respectively. Curve V was calculated by Higashi et al., from the results obtained by freezing method and other thermodynamic methods at 0°C. Curve VI was plotted the results by Katz by means of vapour tension measurement at 25°C.

From Fig. 31 and Fig. 32, the following facts are commonly recognized.

(i) When curve III of "g−a" at 0°C, calculated from the

author's results, was compared with curve V, calculated by Higashi et al., the two curves crossed at the neighbourhood of 0.7 of the value of "a", curve V being situated on the left of curve III at the same water-content "g" above 0.7 of value of "a". Curve V is situated on the right of curve III at the same water-content "g" below 0.7 of the value of "a". As to curve V, when the value of "g" is below 0.5, the value of "a" decreases rapidly with the water-content "g", therefore the angle of inclination of curve V is very gentle in the part of B (0.7∼0.2 of "a").

(ii) When curve IV of "g−a" at 23°C, which was calculated from the author's results, was compared with curve VI, which was obtained by Katz, curve IV agreed with curve VI at above 0.95∼0.98 of the value of "a", and curve of Katz turned away to the right of curve IV at below 0.98 of the value of "a" as seen in every figure.

Considering the fact of (i), in the case of estimation by the freezing method, it is getting difficult to obtain the relation between "g" and "a" at equilibrium state, with depressing of freezing point of the sample according to the decreasing of the water-content "g" due to the non-uniformity of the freezing process of the sample. Therefore, the curve of "g−a" which was obtained by vapour tension method is to be considered perhaps more nearly real than the curve of "g−a" which was obtained by the freezing point determination so far as the range of the value of "a" is small.

If the phenomenon of capillary condensation is taken into consideration respecting curve "g−a" which was obtained by the vapour tension method, the curve of "g−a" which was practically observed will give the smaller value of "a" than the real value of "a" which is shown in the curve of "g−a" of the sample. Therefore, the difference between the curves of "g−a" which were obtained by the vapour tension and the freezing method respectively will become greater.

Briggs[10b] also said that when attempting to determine the water-activity "a" in a system, it is, of course, seldom possible to obtain the accurate values from vapour tension measurements that can be gained from freezing point determination, especially when the activity of the water in the system under consideration, "a", is above 0.9∼0.95, that is not greatly different from that of pure water. However, with the differing of properties of water from that of pure water, the calculated water-activity by freezing point determination is much less accurate than is to be obtained by vapour pressure measurement. The facts in (i) are really recognized.

Next considering the fact of (ii), the difference of curves IV and VI at below 0.98 of water-activity is due to the different mechanism of dehydration or adsorption of water by samples especially by the dehydrating in the author's experiments, and

by both the dehydration and adsorption in the Katz's experiments.

In general, the curve of "g−a" which is obtained by the vapour tension method varies with the difference of the components or hydrogen ion concentration of the protein, or with the different mechanisms of dehydration, or with the adsorption of water, even though the protein is the same. Therefore, curves IV and VI are properly not in agreement. However, when the water-activity is above 0.98, that is to say, as far as the thermodynamic properties of water are not so greatly different from those of pure water, it is recognized that there is no great difference of the observed value with the kinds of the sample. Therefore, curves IV and VI are almost in agreement with each other.

(3) **Conclusion**

Considering the curves of "g−a" for the same sample of gelatine at the same temperature (0°C) which were obtained by vapour tension determination and by the freezing point measurement, both curves cross at the neighbourhood of 0.7 of the water-activity of the sample, but the value of the water-activity "a", which is obtained by the freezing point measurement, is much higher than that by the vapour tension determination at the same water-content "g", when the value of "a" is above 0.7, and "a" of the former is conversely less than that of the latter when the value of "a" is below 0.7. The disagreement of the two curves obtained by the two different measurement due to the non-attainment of equilibrium state by the freezing point measurement when the value of "a" is below 0.7.

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(水產科學研究所業績 第87號)

A STUDY ON THE CORRECTION OF MAGNETIC COMPASS

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(1) Introduction

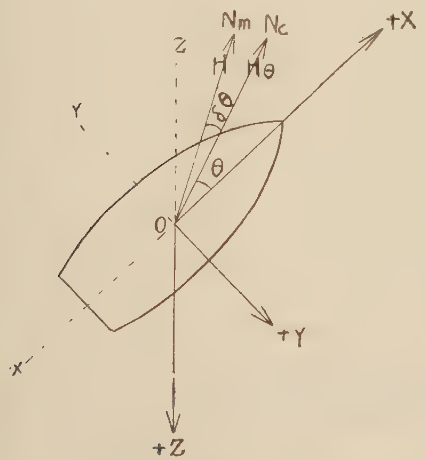
The writer proposed of a method to correct the magnetic compass by the observations of deviations of the compass and directive force of its needles on two or a single heading.

The methods generally followed necessitate keeping ship's head on four cardinal and intercardinal points by compass or magnetic bearing. However, this is a method to correct the compass by ascertaining the coefficients by keeping ship's head on any given two or a single heading.

(2) Method

§ 1. Formulae.

The formulae for this purpose can be as follows:



In figure (above), let X, Y and Z represent the respective axes of ship's fore and aft direction, athwartship direction and vertical direction.

The following Poisson's equation represents ship's magnetic field:

$$\left.\begin{aligned} X' &= X + aX + bY + cZ + P \\ Y' &= Y + dX + eY + fZ + Q \end{aligned}\right\} \quad \text{--- (1)}$$

where: X' and Y' represent the respective components of ship's horizontal magnetic field. a, b, c, d, e and f are the constants induced by the effect of iron structures of ship,—viz. so-called Poisson's Coefficients.

P and Q represent the constants which induce ship's permanent magnetism.

Then let Nc denote Compass North,

Nm : Magnetic North,

H : Horizontal component of Earth's magnetic field,

Z : Vertical component of Earth's magnetic field,

I : Dip,

θ : Compass heading,

δ_θ : Deviation for θ,

and H_θ : Horizontal component of ship's magnetic field.

Then,

$$\begin{cases} X = H\cos(\theta+\delta_\theta) \\ Y = -H\sin(\theta+\delta_\theta) \end{cases} \qquad \begin{cases} X' = H_\theta\cos\theta \\ Y' = -H_\theta\sin\theta \end{cases}$$

and $Z = H\tan I$

Therefore Poisson's equation (1) becomes

$$\left.\begin{aligned} H_\theta\cos\theta &= (1+a)\,H\cos(\theta+\delta_\theta) - bH\sin(\theta+\delta_\theta) + cH\tan I + P \\ -H_\theta\sin\theta &= -(1+e)\,H\sin(\theta+\delta_\theta) + dH\cos(\theta+\delta_\theta) + fH\tan I + Q \end{aligned}\right\} \text{----} \quad (2)$$

Let

$$A_0 = \frac{1}{\lambda}\left(\frac{d-b}{2}\right), \qquad D_0 = \frac{1}{\lambda}\left(\frac{a-e}{2}\right)$$

$$B_\theta = \frac{1}{\lambda}\left(c\tan I + \frac{P}{H}\right), \qquad E_0 = \frac{1}{\lambda}\left(\frac{a+b}{2}\right)$$

$$C_\theta = \frac{1}{\lambda}\left(f\tan I + \frac{Q}{H}\right), \qquad \lambda = 1 + \frac{a+e}{2}$$

Here A_0, B_0, C_0, D_0 and E_0 are called the Exact Coefficients of Deviations, and λ horizontal shielding factor. Generally A_0 and E_0 are very small. Therefore, if A_0 and E_0 are assumed respectively zero, then b and d are regarded zero.

Therefore, (2) becomes

$$\left.\begin{aligned} \frac{H_\theta}{H}\cos\theta &= (1+a)\cos(\theta+\delta_\theta) + \lambda B_0 \\ -\frac{H_\theta}{H}\sin\theta &= -(1+e)\sin(\theta+\delta_\theta) + \lambda C_\theta \end{aligned}\right\} \text{------------------} \quad (3)$$

If a and e are expressed in D_0, (3) becomes

$$\left.\begin{aligned} B_0 &= \frac{H_\theta}{\lambda H}\cos\theta - (1+D_0)\cos(\theta+\delta_\theta) \\ C_0 &= -\frac{H_\theta}{\lambda H}\sin\theta + (1-D_0)\sin(\theta+\delta_\theta) \end{aligned}\right\} \text{------------------} \quad (4)$$

§ 2. Compensation on two headings.

This can be done with a considerable accuracy for directive force on two headings, the only assumption being that the coefficients A_0 and E_0 are zero, which is generally true.

Two headings are available :

a) When a ship is moored alongside of a wharf or pier and afterward winded and moored on the opposite heading or another different heading.

b) When a ship is moored alongside of a wharf or pier, and before and afterward, when she is off them,—for instance entering or leaving harbor.

c) When a ship attained two of her courses at sea. (desirable in the harbor.)

The ship must not, in each case, be near another iron or steel ship or in the immediate vicinity of iron or steel structures on shore.

Now let θ_1 and θ_2 denote two respective ship's compass headings, $\delta_{\theta 1}$ and $\delta_{\theta 2}$ the corresponding deviations for two compass headings θ_1 and θ_2, n a reading shown on the scale when the North point of the compass card was deflected by 45° or 90° by deflector, on shore in a place free from local magnetic influences, and not greatly distant from the ship, n θ_1 and n θ_2 the respective readings shown on the scale for two compass headings θ_1 and θ_2 when the same point was deflected by the same angle by deflector on board the ship in the exact position of the compass needle, and $H_{\theta 1}$ and $H_{\theta 2}$ the respective ship's horizontal magnetic fields for two compass headings.

Then the equation (3) for each compass heading θ_1 and θ_2 becomes respectively,

$$\left.\begin{aligned} \frac{H_{\theta 1}}{H}\cos\theta_1 &= (1+a)\cos(\theta_1+\delta_{\theta 1})+\lambda B_0 \\ \frac{H_{\theta 2}}{H}\cos\theta_2 &= (1+a)\cos(\theta_2+\delta_{\theta 2})+\lambda B_0 \end{aligned}\right\} \text{- - - - - - - - - - - - - - } (5)$$

$$\therefore \quad 1+a=\frac{\frac{H_{\theta 1}}{H}\cos\theta_1-\frac{H_{\theta 2}}{H}\cos\theta_2}{\cos(\theta_1+\delta_{\theta 1})-\cos(\theta_2+\delta_{\theta 2})} \text{- - - - - - - - - - } (6)$$

In like manner,

$$\left.\begin{aligned} -\frac{H_{\theta 1}}{H}\sin\theta_1 &= -(1+e)\sin(\theta_1+\delta_{\theta 1})+\lambda C_0 \\ -\frac{H_{\theta 2}}{H}\sin\theta_2 &= -(1+e)\sin(\theta_2+\delta_{\theta 2})+\lambda C_0 \end{aligned}\right\} \text{- - - - - - - - - - - - - } (7)$$

$$\therefore \quad 1+e=\frac{\frac{H_{\theta 1}}{H}\sin\theta_1-\frac{H_{\theta 2}}{H}\sin\theta_2}{\sin(\theta_1+\delta_{\theta 1})-\sin(\theta_2+\delta_{\theta 2})} \text{- - - - - - - - - - } (8)$$

λ and D_0 are given by

$$\lambda=1+\frac{a+e}{2}=\frac{1}{2}\left\{(1+a)+(1+e)\right\}$$

$$D_0=\frac{1}{\lambda}\left(\frac{a-e}{2}\right)=\frac{1}{\lambda}\left\{\frac{(1+a)-(1+e)}{2}\right\}$$

and from (5) and (7),

$$B_0=\frac{1}{2\lambda}\left[\left(\frac{H_{\theta 1}}{H}\cos\theta_1+\frac{H_{\theta 2}}{H}\cos\theta_2\right)-(1+a)\left\{\cos(\theta_1+\delta_{\theta 1})+\cos(\theta_2+\delta_{\theta 2})\right\}\right] \text{- - - - - - - - - } (9)$$

$$C_0=\frac{-1}{2\lambda}\left[\left(\frac{H_{\theta 1}}{H}\sin\theta_1+\frac{H_{\theta 2}}{H}\sin\theta_2\right)-(1+e)\left\{\sin(\theta_1+\delta_{\theta 1})+\sin(\theta_2+\delta_{\theta 2})\right\}\right] \text{- - - - - - - - - } (10)$$

Then since $\frac{H_\theta}{H}=\frac{n_\theta}{n}$, (9) and (10) can be written

$$B_0=\frac{1}{2\lambda}\left[\left(\frac{n_{\theta 1}}{n}\cos\theta_1+\frac{n_{\theta 2}}{n}\cos\theta_2\right)-(1+a)\left\{\cos(\theta_1+\delta_{\theta 1})+\cos(\theta_2+\delta_{\theta 2})\right\}\right] \qquad (11)$$

$$C_0=\frac{-1}{2\lambda}\left[\left(\frac{n_{\theta 1}}{n}\sin\theta_1+\frac{n_{\theta 2}}{n}\sin\theta_2\right)-(1+e)\left\{\sin(\theta_1+\delta_{\theta 1})+\sin(\theta_2+\delta_{\theta 2})\right\}\right] \qquad (12)$$

Substituting the values of λ, $(1+a)$ and $(1+e)$ into the equations (11) and (12), B_0 and C_0 can be obtained.

Now if the approximate coefficients are expressed by A, B, C, D, E; and the deviation δ_θ for compass heading θ, in degrees,

$$\delta_\theta=A+B\sin\theta+C\cos\theta+D\sin 2\theta+E\cos 2\theta \qquad (13)$$

Then the relation between the exact coefficients and approximate coefficients is as follows:

$$\left.\begin{array}{lll} A_0=\sin A, & B_0\fallingdotseq\sin B, & C_0\fallingdotseq\sin C \\ D_0\fallingdotseq\sin D, & E_0\fallingdotseq\sin E, & E_0=\sin E-\sin A\sin D \end{array}\right\} \qquad (14)$$

Now since A_0 and E_0 are respectively zero, A and E accordingly become zero.

Therefore (13) can be written

$$\delta_\theta=B\sin\theta+C\cos\theta+D\ \text{sind}\ 2\theta \qquad (15)$$

From (14) it follows that

$$B\fallingdotseq B_0\times 57^\circ.3,\quad C\fallingdotseq C_0\times 57^\circ.3,\quad D\fallingdotseq D_0\times 57^\circ.3$$

So that, $B\sin\theta_2\fallingdotseq B_0\sin\theta_2\times 57^\circ.3$ is the amount to be corrected by fore and aft magnets for ship's compass heading θ_2.

$C\cos\theta_2\fallingdotseq C_0\cos\theta_2\times 57^\circ.3$ is the amount to be corrected by athwartship magnets for ship's compass heading θ_2.

$D\sin 2\theta_2\fallingdotseq D_0\sin 2\theta_2\times 57^\circ.3$ is the amount to be corrected by iron spheres for ship's compass heading θ_2.

Example:

When the following data in a certain ship are observed, the compensation on 2nd heading is required.

$\theta_1=45^\circ 45'$ $\quad\delta_{\theta 1}=12^\circ 15'$ E

$\theta_2=245^\circ$ $\quad\delta_{\theta 2}=7^\circ 00'$W

$n_{\theta 1}=22.7$ $\quad n_{\theta 2}=9.1$

$n=17.8$

Using the equations (6), (8), (11) and (12), the following values are obtained:

$1+a=1.043 \quad \lambda=0.927 \quad D_0=+0.125 \quad C_0=-0.2445.$

$1+e=0.811 \qquad\qquad\qquad B_0=+0.365$

Then the amounts to be corrected on ship's compass heading 245° are,

$B \sin \theta_2 \fallingdotseq B_0 \sin \theta_2 \times 57.°3 \fallingdotseq -18.°9$

$C \cos \theta_2 \fallingdotseq C_0 \cos \theta_2 \times 57.°3 \fallingdotseq +5.9$

$D \sin 2\theta_2 \fallingdotseq D_2 \sin 2\theta_2 \times 57.°3 \fallingdotseq +5.5$

Then of these values, $-18.°9$ due to B_0 is to be compensated by fore and aft magnets, $+5.°9$ due to C_0 by athwartship magnets and $+5.°5$ due to D_0 by iron spheres.

§ 3. Compensation on one heading

Should it not be possible to take observations for directive force on two headings, the compass can be corrected with tolerable accuracy from observations on one heading only. Center the compass accurately, place the spheres by estimate, and then compensate the heeling error regardless of the fact that the ship may not be heading near East or West.

Next, record the compass heading of the ship by compass to be corrected. And observe and record the magnetic heading obtainable by means of a chart, or an azimuth of the sun, or of a distant object of known magnetic bearing, or by a single set of reciprocal bearings.

Let θ denote ship's compass heading,

δ_θ : deviation for θ

n : a reading shown on the scale when the North point of the compass card was deflected by 45° or 90° by deflector, on shore in a place free from local magnetic influences, and not greatly distant from the ship

n_θ : a reading shown on the scale for ship's compass heading θ when the same point was deflected by the same angle by deflector on board the ship in the exact position of the compass needle

And then using the equation (4), B_0 and C_0 are obtained as follows:

$$\left.\begin{aligned} B_0 &= \frac{1}{\lambda}\frac{n_\theta}{n}\cos\theta-(1+D_0)\cos(\theta+\delta_\theta) \\ C_0 &= \frac{-1}{\lambda}\frac{n_\theta}{n}\sin\theta+(1-D_0)\sin(\theta+\delta_\theta) \end{aligned}\right\} \text{- - - - - - - - - - - - - -} \quad (16)$$

In this event the quadrantal spheres must be placed by estimate and values of λ and D_0 assumed; the value of λ, being generally 0.70 to 0.95 average, may be assumed as approximately 0.9 when λ cannot be observed. In the same manner as in the compensation on two headings, B_0, C_0 and D_0 can be expressed by B, C and D, and the deviation may be compensated on any compass heading as desired. Viz —

Let $D \sin 2\theta \fallingdotseq D_0 \sin 2\theta \times 57.°3$ be the amount to have been compensated by iron spheres assuming the values of D_0, and then $B \sin\theta \fallingdotseq B_0 \sin\theta \times 57.°3$ is the amount to be compensated by fore and aft magnets, and $C \cos\theta \fallingdotseq C_0 \cos\theta \times 57.°3$ the amount to be compensated by athwartship magnets.

Example:

From data for standard compass of a similar ship, assumed: And estimated that the deviation due to D_0 has been compensated by iron spheres. It is then found that:

$\theta = 21°$ $\delta_\theta = 5°10'W$

$n = 25.3$ $n_\theta = 14.8$

Compensate on ship's compass heading 21.°

Using (16), the following values are obtained:

$B_0 = -0.35.$ $C_0 = +0.04$

Therefore the amount to be compensated on compass heading are,

$$B \sin\theta \fallingdotseq B_0 \sin\theta \times 57.°3 \fallingdotseq -7.°2$$

$$C \cos\theta \fallingdotseq C_0 \cos\theta \times 57.3 \fallingdotseq +2.1$$

Of these values, $-7.°2$ is to be compensated by fore and aft magnets, and $+2.°1$ by athwartship magnets.

In such cases as described above in the compensations on two or a single heading, the computed values of these portions $B \sin\theta + C \cos\theta$ may differ appreciably from the actual values of δ_θ; this is due to the reason that the relation between the Approximate and Exact Coefficients of Deviations in (13) has been required approximately.

§ 4. Analysis of Coefficient B_0

As the analysis of coefficient B_0 was described in the previous report by the writer, this subjest is herein omitted.

(3) Conclusion

The writer considers that this may be good enough to adopt as a method to correct the compass on two or a single heading in taking advantage of the time when a ship is being moored or lying in the harbor.

As the coefficients A_0 and E_0 are supposed zero and the formulae used in computations are obtained approximately, this cannot be called an absolutely perfect method. However, the writer believes that this is well adopted for the practical use of magnetic compass correction, and navigators are earnestly requested to pay attention to this work and to try this method at sea.

（水產科學研究所業績　第88號）

貝類棲息地の環境條件に關する研究（第3報）

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STUDIES ON THE ENVIRONMENTAL CONDITIONS OF SHELLFISH-FIELDS.

III. IN THE CASE OF MURORAN HARBOUR (2)

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Herein is presented the results of the 4th investigation, which takes into consideration the oceanographical conditions pertaining to the shellfish-field of Muroran, Hokkaido. The paper is based upon the summary of the results of the 4 investigations hitherto undertaken.

Owing to the fact that the 4th research was prosecuted in summer, distinct seasonal characteristics could be observed in the results as on the vertical distribution or on the contents of salinity, water temperature, dissolved oxygen, or other nutritive salts contained in the water.

The water properties, especially respecting the soluble organic matters, phosphate and nitrite, are discussed in full and the summer conditions are compared with the winter. The contents of soluble organic matters in the harbour water in winter showed 1.4 times that of the summer; the difference between the surface and the bottom layers was very small. In summer the phosphate contents of the surface layer was larger than the bottom and the range between the two layers was large. The phosphate content in summer was about $1/5.2$ that of the winter and the amount contained in the surface layer is smaller than in the bottom layer the range is large in winter. The nitrite content was large in winter as in the case of the former two, being 11.4 times larger than in summer; the content in the surface layer was larger than that of the bottom, but in summer the contrary is the case. Judging from the relation between nitrite and phosphate contents in the harbour, it seems that the former is more important than the latter.

Every investigation on the bottom deposits yielded nearly the same results, but owing to the quantity of mineral crusts or coal waste which were thrown out of iron works or ships, the properties of the bottom material are somewhat changeable.

Plankton survey was carried out only once during the summer. The composition of

species was nearly the same as that of the North-West of the same season. The degree of pollution in the harbour could not be determined by means of the plankton survey, because there was no "Red-water" at the collecting time.

1. 緒　　　　言

函館港の海況及び水質の調査と港の現状を調査し、同港に於て増殖している貝類、殊にホタテガイの種苗保護上の海況条件の変化を明かにすると共に、港湾内に流入する汚水及び河川より流入する汚水、汚水の貝類増殖に及ぼす影響をも併せて究明するため、昭和24～25年にわたり4回の調査を実施したが、そのうち第3回までの調査結果については既報[1]に於て報告した。この第1回の調査中、水質の調査を行った第2回（昭和24年11月）及び第3回（昭和25年2月）の調査時は、いずれも冬の水温低期に当っており、調査港を全般として把握するためには不備であるから、この点を補足究明する意味に於いて、第4回は夏季の盛夏期をとらえ、25年8月3日に調査を行った。本報に於てはこの第4回の調査を中心として、前3回の調査結果を併せ比較検討し、函館港における海況変化の貝類増殖に及ぼす影響を総合的に考察した結果を報告する。

本調査研究にあたり[illegible]及びプランクトンの[illegible]に深く謝意を表すると共に、調査費は[illegible]の一部を使用したものであることを記して感謝の意を表す。

2. 調　査　方　法

調査地点は第3回の調査時とほぼ同様の12地点をとり（第1図）水質、底質及びプランクトンについて調査を行った。

採水には北原式B号採水器を用い、表層、5m層及び底層の採水を行い、底質にはエクマン採泥器及び北原式採泥器を使用した。プランクトンは北原式定量用ネット（XX13）を使用して、各地点に於て底より表面までの垂直採集を行った。

水質については水温、比重（σ15）透明度、塩分、溶存酸素、硫化水素、化学的酸素要求量、燐酸塩、珪酸塩及び亜硝酸塩などの分析測定を行い、底質については硫化物含量、灼熱減量、有機炭素及び全窒素量を定量測定したが、その方法に関しては前報と同様である。プランクトンについては定性的に検鏡を行ったものである。

3. 調　査　結　果

内湾に於ける海流は主として干満潮に基く潮汐と吹送、風の影響によるもので、海況の調査を行うに当っては、常にそれらの影響を考慮しなければならない。冬季循環期においての3回の調査と、夏季の停滞期の第4回の調査結果を比較すると、それぞれの特徴は種々の点において見られる。以下に各成分についての結果を略述する。

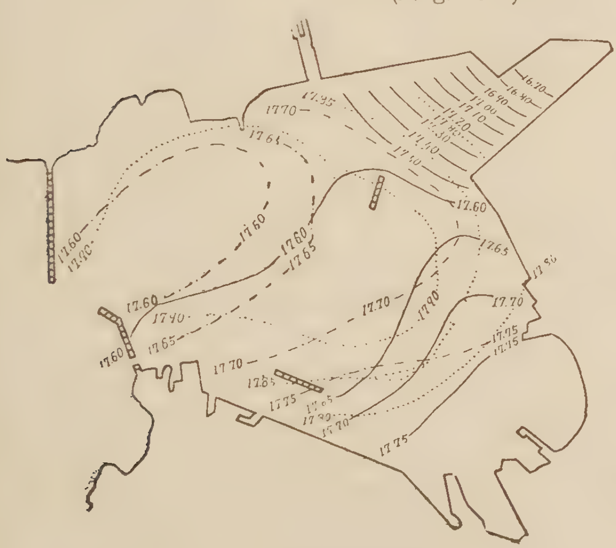
I. 水　　　質

第4回の水質に關する調査結果は第1表の通りである。

Table 1. Properties of Sea Water in Muroran Harbour (Aug. 8, 1950)

Station No.	Depth of the Observed Layer(m.)	Water temp. (°C)	pH	Cl (‰)	O_2 (c.c./L.)	Soluble organic matter (mg/L)	H_2S mg/L)	P_2O_5 (mg/m³)	SiO_2 (mg/m³)	Nitrite (mg/m³)	Fe (mg/L)
1	0	24.0	8.4	16.73	4.69	9.35	0.30	15.1	2310	2.5	0.28
	5	22.4	8.3	17.71	4.54	7.78	0.05	12.1	tr.	1.2	0.23
	9	21.9	8.1	17.71	3.53	5.99	0.26	19.2	2595	2.8	0.30
2	0	24.2	8.4	17.58	5.64	7.54	0.30	tr.	1170	0.4	0.22
	5	23.0	8.4	17.68	5.49	7.35	0.07	tr.	tr.	tr.	0.22
	9.5	21.5	8.2	17.87	3.27	7.65	0.44	34.3	1860	3.9	0.13
3	0	24.1	8.4	17.66	6.01	6.55	1.10	18.7	tr.	1.2	0.27
	5	23.2	8.4	17.69	5.59	9.54	0.16	tr.	tr.	0.5	0.21
	9.5	21.3	8.1	17.89	2.69	8.81	0.16	8.5	2358	tr.	0.13
4	0	24.2	8.4	17.75	6.14	9.19	0.25	tr.	1048	0.3	1.68
	5	22.8	8.3	17.73	4.61	9.07	0.26	2.0	4900	tr.	0.24
	8.5	21.4	8.0	17.78	2.26	4.21	0.14	17.1	2448	4.4	0.25
6	0	24.4	8.4	17.61	6.30	4.10	0.30	17.1	3065	2.5	0.18
	5	23.6	8.4	17.67	6.44	5.99	0.27	17.1	2818	tr.	1.26
	12.5	21.3	8.2	17.96	4.20	5.37	0.90	tr.	1857	tr.	0.23
7	0	24.4	8.4	17.63	6.66	6.64	0.03	tr.	tr.	0.3	0.27
	5	23.2	8.4	17.79	6.07	6.12	0.19	tr.	tr.	tr.	0.22
	9.5	21.9	8.1	17.86	4.37	4.92	0.67	28.1	tr.	tr.	0.23
8	0	24.7	8.4	17.52	6.34	7.84	0.26	19.8	3125	0.6	0.34
	5	24.6	8.4	17.53	6.54	6.27	0.25	7.1	1133	34.5	0.32
	9.5	22.8	8.3	17.89	5.31	6.34	0.29	tr.	tr.	1.8	0.32
9	0	24.5	8.4	17.53	6.07	7.65	0.16	8.0	tr.	0.3	0.21
	5	23.7	8.4	17.59	5.80	9.43	0.35	tr.	2500	12.9	0.33
	12.5	21.4	8.2	18.00	4.24	9.04	0.22	17.1	2650	tr.	0.14
10	0	24.8	8.4	17.63	7.41	9.73	0.30	26.0	tr.	tr.	0.03
	5	23.9	8.4	17.64	6.01	6.03	0.18	51.2	tr.	2.1	0.16
	10	22.4	8.3	17.84	4.12	4.32	0.32	52.1	tr.	2.6	0.28
11	0	23.4	8.4	17.70	5.25	4.17	0.13	27.4	tr.	0.1	0.18
	5	22.5	8.4	17.75	5.33	5.39	0.17	17.1	tr.	tr.	0.19
	13	21.6	8.3	17.98	4.91	10.01	0.20	0.20	1753	1.1	0.22

Fig 1. Horizontal Distribution of Cl (‰) (Aug. 1950)

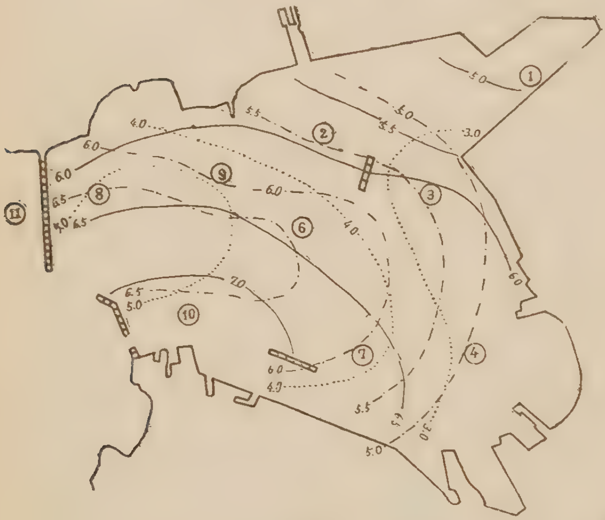


Surface Layer (solid line)
5Meter Layer (dashed line)
Bottom Layer (dotted line)

(1) 水溫……第2回(11月)、第3回(2月)の兩調査時に於ては、表層、底層がほとんど同溫度で循環期にあることを示していたが、この第4回の調査においては底層に行くに従つて水溫が降下し、明かに夏季停滯期になつていることが知られる。水平的な溫度の分布狀態については、各回を通じて特記すべき差異は認められない。

(2) 塩分……總體的にみて、冬季においては塩分の垂直分布には殆ど變化がみられなかつたが、夏季においては表層は塩分の低い水で被われ、底層に進むにつれて塩分の高い港外水が入り込んでおり、冬季のような循環流は夏季には形成されず、それぞれの時期の特徴がみられる(第1圖)。

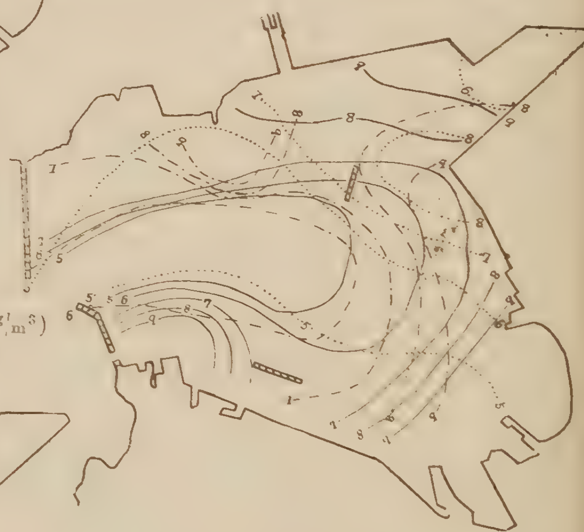
Fig 2. Stations of Observation in Muroran Harbour, and Horizontal Distribution of O_2 (c.c./L) (Aug. 1950)



——— Surface Layer
- - - - 5Meter Layer
.... Bottom Layer

港外水（塩素量18.00‰前後）の港内への影響をみると、冬季の調査時と、ほゞ同様に、港口底層より北東に入り、本輪西地先に向けて進入し、それが南下して港南部の市營埠頭まで到達し、更に港東部においても、日鐵埠頭近くまで底層へ進入しているのがみられる。また冬季にみられた市營埠頭附近の停滞した汚水はこの時にはみられず、日鐵埠頭附近表層には前回と同様汚水の停滞があり、それが港北部一帯の表中層にまで擴がつている。

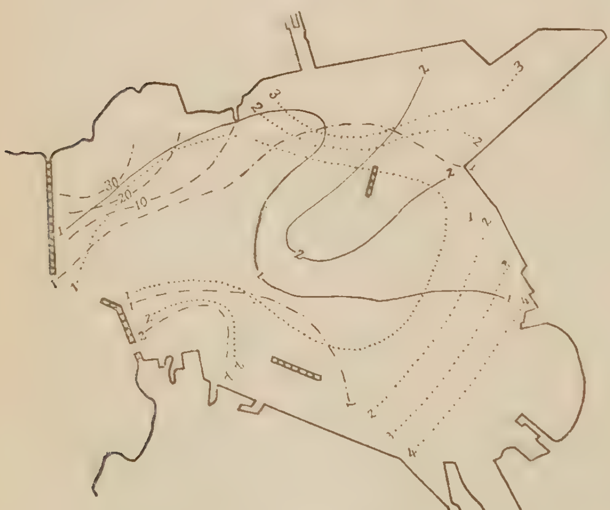
Fig 3. Horizontal Distribution of Soluble Organic Matter (mg/L) (Aug. 1950)



——— Surface Layer
- - - - 5 Meter Layer
.... Bottom Layer

(3) 溶存酸素……塩分の分布の場合にみられたと同様に、冬季には表層と底層との著しい變化はないが、夏季の場合には明かに底層にいたるに從つて漸次溶存酸素の減少していることが知られる（第2圖）。汚染水の入りこむと思われる日鐵埠頭附近、御崎埠頭附近並びに市營埠頭

Fig 4. Horizontal Distribution of NO_2 (mg/m³) (Aug. 1950)



——— Surface Layer
- - - - 5 Meter Layer
.... Bottom Layer

Fig 5. Horizontal Distribution of P_2O_5 (mg/m³) (Aug. 1950)

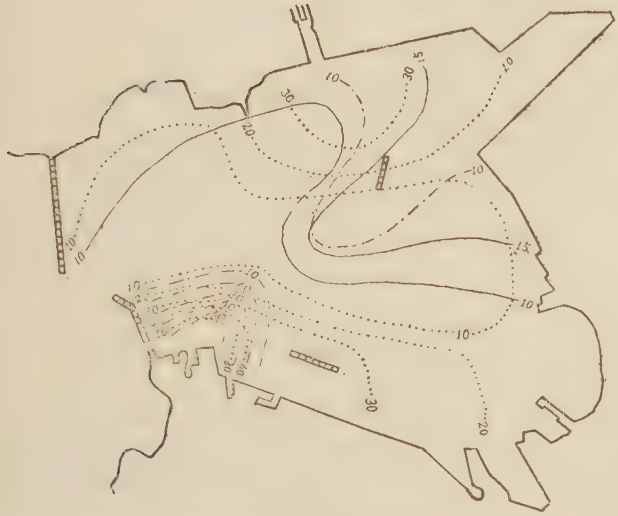
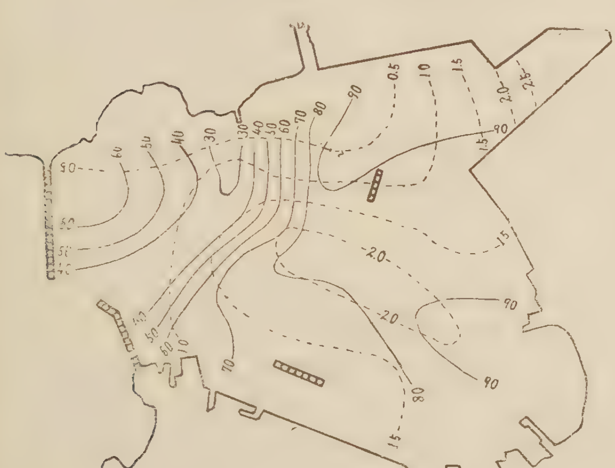


Fig 6. Distribution of Mud in Fine Soil and Organic Carbon Contents in Bottom Materials (%) (Aug. 1950)



附近（St. 1, 2, 3, 4）では、溶存酸素量は減少の傾向がみられる。又、底層水の酸素含有量は底質の粒度組成と負の相關關係にあり、泥量の多い地點では酸素量の減少しているのも興味がある。

(4) 可溶性有機物……可溶性有機物は海水汚染の一つの指標として他の要素と共に重要なものであるが、冬季に比べると夏季は各地點ともその含量が少くなつている。その分布狀態は酸素の分布狀態とは負相關的で、汚染水の影響を示すものと想われる。ドック沖（St. 10）には有機物含量の多い水塊が冬季の調査時にみられたが、夏季の場合にも出現していることは注目すべきことである(第3圖)。

(5) 亞硝酸塩……港内水は港外水に比して一般にその含量が多く、而かも港奥部程多くなつている。これは港內へ流入する都市汚水から供給されることを示すものと考えられる。冬季に比べて夏季は著しく減少しているが、それはプランクトンの增殖によつて消費されたものと思われる（第4圖)。

(6) 燐酸塩……亞硝酸、塩硅酸塩と共に、冬季に比べて甚だしく減少し、夏季停滯期の特徵を示している。たゞドツク沖(St. 10）の地點では比較的含量が多くなつている。これは防波堤が根元で切斷されているので、その部分から燐酸塩の多い港外水が流入した結果であろう（第5圖)。

(7) 硅酸塩……亞硝酸塩の場合にみられたように、一般に港外水に比してその含量は多くなつているが、これは亞硝酸塩と同樣、都市汚水の流入によるものであろう。しかも汚水の影響を強く受ける地域ではその含量が他よりも多くなつている。また他の榮養塩と同樣冬季に比して著しく減少しているのが目立つている。

(8) 硫化水素……冬季に比べて著しい差異は認められないが、港の中央部(St. 6)の底層と日鐵鑛碎投棄場沖合（St. 3）の表層に夫々 0.9mg/L, 1.1mg/L という含量の多い部分がみられる。しかし一般的

に含有量から考えれば、室蘭港では硫化水素が貝類の棲息に影響を及ぼしているとは思われない。

Ⅱ. 底　　　質

第4回の調査の底質に關する分析結果は第2表の通りである。

Table 2. Properties of Bottom Materials in Muroran Harbour (Aug. 8, 1950)

Station No.	Depth (m.)	Igniton Loss (%)	Orgainc Carbon (%)	Total Nitrogen (%)	Gravel in Original Sample (3mm<) (%)	Mud in Fine Soil (0.05 mm>) (%)	Remarks
1	9.0	10.22	2.75	0.16	2.38	96.32	
2	9.5	3.84	0.43	0.04	8.56	95.92	Some coal wastes.
3	9.5	5.61	1.18	0.04	1.19	81.54	Many pieces of mineral crusts
4	8.5	8.15	1.95	0.10	0.34	97.24	
6	12.5	9.83	2.14	0.12	0.69	85.04	
7	9.5	6.67	1.46	0.06	4.53	71.22	Fine sand 23%.
8	9.5	6.68	0 65	0.07	15.97	68.66	Some coal wastes and gravels
9	12.5	6.93	1.34	0.11	31.79	28.79	Many coal wastes.
10	10.0	7.07	1.40	0.09	4.26	68.66	Sand 21%.

(1) 粒度組成……粒度組成は前3回の調査結果とほゞ同様であるが、本輪西沖（St. 9）に異常に礫の多いところがあり、又 St. 7 も礫の割合が増している。これは浚渫あるいは炭砕投棄などにより底質自體が變化したためか、或は採泥の不備によるものか詳かではないが、採泥器によつて得られた3mm 以上の礫の大部分が石炭砕であつたこと、及び該地點が船舶の投錨地にあたつていることなどから考え、船舶からの投棄物に原因するものと推察される。總じて港奥部は泥質で、港口に向うにつれて漸次砂泥質となつており、その分布は港内流の勢力、從つて港内水の諸性質と密接な關連性がみられる。又港東部の日鐵地先（St. 2）では他の港奥部の底質組成に比して割合に粗い組成を示しているのは、同地附近における鑛砕投棄によるものと思われる（第6圖）。

(2) 有機炭素並に灼熱減量……兩者は共に類似した分布を形成しており、港奥部の水の比較的停滯しがちな附近と、港の中央部の割合に深い港凹部では共に多くなつている。冬、夏兩期についてみれば、全體として著しい季節的變化はみられないが、灼熱減量は幾分夏季に於て増加しているようである（第6圖）。

(3) 全窒素……前回の調査と大差なく、有機炭素並に灼熱減量の分布と同様、港奥部（St. 1, 4）及び港中央部（St 6）に含量の多い部分がみられる。しかし季節的消長は殆どみられない。

Ⅲ. プランクトン

各調査地點におけるプランクトンの出現狀況は第3表の通りで、この調査の結果では、港内各地點による著しい差は認められず、腹足類や辦鰓類などの貝類幼生も殆ど各地點においてみられている。即ちこの調査に於てはプランクトンによつて港内の汚染度を知ることはできなかつた。出現種類は大體同時期の噴火灣調査結果[2]と比較すると殆ど共通であつて、特殊なものはみられなかつた。

前3回の調査においてはプランクトンの採集を行わなかつたので、冬季と夏季による相違をみることはできない。又、第4回の調査時は「赤水」といわれる所謂日鐵の廢水の殆ど無かつた時であるから、赤水の放出された時期、或はその少し後の時期に於てプランクトンの調査を行い、平時と比較検討することは、工場廢水の影響を調べる上に極めて有意義なことゝ思われる。

4. 考　　　察

室蘭港は割合狹隘な港口をもつて噴火灣に連り、海底形態も著しい特徴はみられず、港口及び港の

Table 3. Plankton in [illegible] Harbour

Station No.	1	2	3	4	6	8	9	10	11
1. [illegible]						rr			
2. [illegible] sp.			r					rr	
3. Bellerochea malleus			rr				rr		
4. Biddulphia pulchella							rr		
5. Campylodiscus [illegible]						rr			
6. Chaetoceros affine	–	+	–	–	+	+	+	–	–
7. Ch. [illegible]	r	r			r	r	r	r	r
8. Ch. decipiens	r	r	r	r	r	r	r	r	r
9. Ch. didymum	r		r	r	r	r	r	r	r
10. Coscinodiscus [illegible]		rr	rr			rr	rr		
11. [illegible]		rr							
12. Leptocylindrus [illegible]			r	r	r		r	r	
13. Melosira Borreri	r	+	+	r	r	r		rr	rr
14. Navicula [illegible]			rr				rr		
15. [illegible]	r		r	r		rr		rr	
16. N. seriata	r	r	r	r	r	r	r	r	r
17. Rhizosolenia [illegible]	r	r		r		rr	rr	rr	r
18. Rh. [illegible] f. semispina			r		rr	r		r	r
19. Skeletonema costatum	r	r							
20. Thalassiothrix Frauenfeldii	r				rr	rr		rr	
21. Th. nitzschioides	–	+	+	r	r	r	r	r	r
1. Ceratium Fusus	r	rr	rr	rr	rr	rr	rr	rr	rr
2. C. furca	r	rr	rr	rr	rr		rr	rr	rr
3. C. macroceros	r	r	r	r	r	r	r	r	r
4. C. tripos	c	+	+	r	+	–	+	+	+
5. Noctiluca scintillans	–	r	cc	r	r	cc	r	r	+
6. Peridinium depressum	rr	rr	rr	rr		rr		rr	rr
7. [illegible]	rr								
8. Prorocentrum [illegible]	r	r	rr	rr					rr
9. Favella [illegible]	–	–	–		+		r	–	–
10. [illegible]			rr				r		
11. Tintinnopsis [illegible]						rr			
12. T. [illegible]		rr	rr	rr	rr	rr	rr	rr	rr
13. [illegible]			rr						
14. Obelia sp.			rr		rr	rr			
15. [illegible] sp.						rr			
16. [illegible] sp.		rr			rr	r		r	
17. [illegible]			rr		c			–	
18. Oithona nana	–	c	+	–	+	r	+	rr	+
19. O. similis	cc	–	c	c		+			c
20. Paracalanus parvus	c	cc	cc	cc	c	c	r	c	cc
21. [illegible]		rr	+	+	rr				r
22. Nauplius larva		r	r	r	r	rr	rr	r	r
23. [illegible]			+	–	–	+		rr	
24. Podon polyphemoides	c	cc	cc	cc	cc	c	c	c	+
25. Microsetella norvegica			c		r	rr		rr	
26. [illegible]		rr	rr						
27. [illegible]	r	c	c	+	c	c	r	+	+
28. Zoea larva						rr			
29. [illegible] larva	rr								
30. Polychaeta larva	rr	rr	rr	rr	rr		rr	rr	
31. Gastropoda larva	rr	rr	rr			rr	rr		rr
32. Lamellibranchia larva	r	r	r	r	r	rr	rr		rr
33. Sagitta sp.			–	rr					
34. [illegible] sp.			r			–		r	
35. [illegible]				rr					

中央部が比較的深く（12～16m）、港奥防波堤の東北部 St.2 とSt.1 の中間邊に干潮時に露出する砂洲が存在するが、その他は余り起伏なく10m以下となつている。

室蘭における有用貝類としては、ホツキガイとホタテガイが擧げられるが、前者は港外に棲息し、港口南部にあたる祝津は特に多數の稚貝が發生するので知られている。ホタテガイは室蘭港口附近のみならず、港内にも多數棲息し、4月16日より6月末日に至る禁漁期をのぞいては、港の内外に於て多數の漁船がその採取に従事している。港内には更にアサリその他二、三の食用貝類がみられるが、それらの産額は前者に比して極めて少い。港内に於けるこれら有用貝類の棲息地は、ホタテガイは港の東奥より西北港口方面に多くみられアサリは北東の本輪西方面に多い。ホタテガイの最近の産額は23,000～39,000貫であるが、昭和4～8年の平均年産額86,300貫に比べると非常に少くなつてきている。又アサリは大正11～15年までの年平均産額は約10,000貫で、當時のホタテガイの産額の1/2にも達していなかつた。しかもアサリは逐年産額が減少してきているものゝ如く、最近ではアサリ單獨の漁獲統計がみられないという狀態である。このように漁獲が減少してきた原因を明かにすることは極めて困難なことであるが、貝類棲息地の環境の現狀を把握することは今後の生産に重要な意義を有するものであるから、これまで行つた4回の調査結果に基いて、室蘭港における貝棲息地の環境狀況について考察を加えてみる。

前報において指摘した如く、室蘭港の海洋學的條件を支配するものは、港口底層より時計廻りに港内深く進入している港外水と、港奥に停滯している工業地帶及び市街地より入る汚水の混つた低鹹水塊との平衡であり、それが港内の生物や底質との關連において港内における物質の代謝循環系をなしている。

第4表は冬季と夏季について夫々表層、底層に別け、測定値の平均値をとつて可溶性有機物、燐酸塩並に亞硝酸塩の季節的變化を示したものである。

Table 4. Seasonal Changes of Nutritious Matters of Sea Water in Muroran Harbour

Classification \ Nutritious Matter			Soluble Organic Matter (mg/L)	P_2O_5 (mg/m³)	P (mg/m³)	NO_2 (mg/m³)	N (mg/m³)
Feb.	Inner	Surface Water	9.13	59.7	13.0	17.2	5.2
		Bottom Water	9.52	103.7	22.5	12.3	3.7
		Average (A)	9.33	81.7	17.8	14.8	4.5
	Outer	Surface Water	7.36	65.7	14.3	6.6	2.0
		Bottom Water	7.04	181.3	39.5	18.1	5.5
		Average (B)	7.20	123.5	26.9	12.4	3.8
	Ratio of Inner to Outer (A)/(B)		1.3	1/1.5		1.2	
Aug.	Inner	Surface Water	7.62	11.6	2.5	0.9	0.27
		Bottom Water	5.96	19.6	4.3	1.7	0.52
		Average (C)	6.79	15.6	3.4	1.3	0.40
	Outer	Surface Water	4.17	27.4	6.0	0.1	0.03
		Bottom Water	10.01	44.4	9.7	1.1	0.33
		Average (D)	7.09	35.9	7.9	0.6	0.18
	Ratio of Inner to Outer (C)/(D)		1/1.04	1/2.3		2.2	
Seasonal Change on the Whole Harbour (A)/(C)			1.4	5.2		11.4	

これら3榮養物質は夏季は冬季に比して著しく減少しており、冬と夏とによる海況の特徴があらわれている。可溶性有機物は冬季が夏季よりも多く、夏季の1.4倍となつており、しかも冬季には底層が表層より僅かに多いが夏季には逆に表層が底層よりも多くなつていて、その偏差も大きい。これは冬季は循環期にあり、海底面に沈降していた微細泥並に有機細片が攪亂されて浮游し、一部可溶性となつたために、底層の含量

が増加する結果となつたものであろう。これに反し夏季は停滞期であり、循環流がなく、底層流も緩漫となり、海況が極めて安定となつているために微細泥の攪亂もなく、従つて冬季の様な底層に於ける可溶性有機物の増加は認められず、表層は流入せる汚水の影響により幾分大きい値を示しているものであろうと考えられる。港外表層水は流入水の影響が極めて少いので、その可溶性有機物含量は少くなつている。

燐酸塩についてみれば、全般的に夏季には冬季の1/5.2に減少しており、冬季夏季を通じて表層に少く底層に多くみられ、偏差は冬季の方が大きい結果となつている。冬季間多量に存在していた燐酸塩は、春季のプランクトン繁殖期にあたつて急激に消費され、その補給が續かないために夏季は少くなり、また表層に於ては生物は潤澤なる日光、酸素の供給をうけて榮養物質を水中より同化して自己の體を形成し、或はそのエネルギーを利用して活動するが、底層では表層に比して光が充分に達しないため同化作用が鈍り、その結果、表層では燐酸塩の消耗が多く底層は少いということになり、これが調査結果にも反映したものと想われる。港内水の燐酸塩の含量が港外のそれよりも少いことは、燐酸が日鐵其他より排出される工場水に含まれる鐵分と化合し、燐酸鐵として沈降してしまうためであろう。

亞硝酸塩も前2者と同様、冬季に多く夏季に少く、且つその偏差は極めて大きく11.4倍となつている。冬季に於て表層が底層より多くなつているのは、表層を被うている汚水中に含有するアンモニアが硝化細菌によつて酸化され NO_2、NO_3 が合成されたためであろう。一方夏季に於ては逆に底層が表層に比して増加しているのは、燐酸塩の場合と同様、表層における生物の生活活動も底層におけるよりも活潑で、窒素が表層においてより多く消費されるためと思われる。また冬季、夏季を通じて港外水よりも港内水の NO_2 量が多くなつているのは港内に流入する汚水の影響を反映しているためであろう。

底質の理化學的諸性状は、港内水の種々な特性とは極めて強い相關を示すものであり、室蘭港に於ける物質循環系の一要素として重要な役割を果している底質の化學組成の消長は看過することができないし、港内生産力にも大きな役割を果しているものと考えられる。しかし底質に關しては前回調査の資料が少いので、夏季の場合との比較検討が困難である。

上記3榮養物質は室蘭港では生産に興え重要な物質であるが、燐酸塩及び亞硝酸塩について更に少しく検討してみる。

海水中の硝酸態窒素と無機態可溶性燐の關係については、Fleming[2]等によると、その間には濃度に關係なく、ある範圍内で一定の重量比が存在し、海水中に存在する有機體についても同様のことが報告されている。かゝる觀點から谷井等[1]は稲田中の窒素と燐の季節的變化を明かにし、N:P=7.2:1の比を満足するPの量を求め、その結果から稲田中の生物量の消長の理由を究明している。

室蘭港の場合についてNとPの關係をみると、冬季夏季を通じて窒素量が遙かに小さい値を示しており（勿論この場合は NO_2 態のNだけしか測定していない。NO_3 態のNをも測定して、それらについて考察しなければ妥當とはいえないが）、また夏季には冬季に對しNは1/11.4、Pは1/5.2に減少しているなどの點からみて、本港に於ては燐よりも窒素の方が生産を支配する最小物質となつている様に見えるが、本港の場合の様に外部から供給される物質の種類とその量の複雑な場所に於ては、簡單に測斷することは許されない。

室蘭港は前述の如く、港口から進入した港外水は港内全域に及び、工業地帯並びに市街地から流入する汚水を稀釋し、風や水温の變化に伴つて生ずる循環流とともに汚染水の浄化にあづかつている。従つて、一般的にいつて、わが國屈指の工業地帯をなすこの市街地から相當量の汚水が流入し、また、

出入する多数の船舶により汚染されている本港の一部に、ホタテガイやアサリの棲息していることは一見奇異なことであるが、これは外洋水などの大きな浄化作用によるものであり、港外水の出入の消長が貝類の棲息にあづかつて大きな力があるものと考えられる。

ホタテガイの棲息條件については Dakin[5]、今井[6]、木下等[7]は主として砂泥及び礫質の安定な海底に分布が濃密であることを指摘し、また西岡・山本[8]はホタテガイの分布を決定するものは底質特にその泥粒組成のみならず、それ以外の海底條件が重要な因子であることを報告し、更に木下等[9]はホタテガイの生活環境としては潮流の比較的強いことが必要條件で、底質はむしろ二次的附帶條件であることを報告している。

室蘭港の現在のホタテガイ棲息場は底質の粒度組成とよく關連し、港北部の礫の多い地域を主漁場として榮地町沖合から港口にかけ、泥量80%の等泥粒線北西にあたつている。この地域は底質からも知られる如く、比較的港外水の影響のうけやすい、所謂潮通しの良好なところである。しかし泥量80%以上の等泥粒内でもSt. 2、3、6附近には少しく棲息がみられる。このように室蘭港の場合、相當程度の密泥地域にも僅かながらホタテガイが棲息してはいるが、棲息量の少いところからして、多くの人々の指摘しているようにホタテガイは密泥地域には適さないようである。

西岡・山本・野村等[10]はホタテガイの稚貝がたとえ密泥地域に散布されても、その稚貝が健全であるならば、決して斃死するものではないが、底質攪亂によつて生ずる浮游泥が斃死を招來するものであることを報告しており、また同様に倉茂[11]はアサリについて、浮游泥が多量の場合には斃死することを述べている。

浮游泥の貝類に及ぼす影響は、たゞ單に浮游泥が貝類の水管その他の部分から入つて鰓に附着し、機械的にその呼吸、攝餌作用に障害を與えるだけではないように考えられる。著者等[12]は海底上による海水溶存酸素量の消費に關する實驗に於て、底質の泥粒組成と溶存酸素消費量との間には正の相關が存在することをみており、浮游泥が溶存酸素を消費し、或は榮養物質を吸着し、更にまた、有機物質を可溶性となすなど、間接的にも貝類棲息にとつて支障を與えるであろうことは想像に難くない。これらの事柄については夏季の調査結果によつても推察される。即ち、底層の酸素分布は底質の泥粒組成と負相關を示し、濃密泥地域では溶存酸素量が減少しており、底質並びに浮游泥により消費されたものと思われ、又可溶性有機物は夏季は底層が小さい值を示しているに反し、冬季には含量が增加しており、冬季循環期にあたつて底層攪亂により、底質若しくは浮游泥を媒介として有機物が可溶性となつたためであろうと考えられる。このように密泥地域は底層の攪亂によつて浮游泥を伴い、貝類の棲息に悪環境を形成するものゝようである。

第3回（冬季）調査の直前におこつた港内ホタテガイの斃死に關しては、前報においても推論した如く、斃死發生の前は北東の風が烈しく、港内水が攪亂され、その結果港奥の低鹹水と港外水との平衡狀態が破られ、底層の攪亂も著しいために沈降していた多量の微細泥が浮游し、それが直接間接にホタテガイの斃死に拍車をかけたことも大きな原因の一であると考えられる。

5. 摘　　要

本報は前報に引續き室蘭港の第4回の調査結果について報告し、且つ前後4回にわたる調査結果を總括的に比較検討し、室蘭港の貝類棲息地としての海洋學的特性について考察したものである。

第4回の調査は夏季にあたり、前回の冬季の結果と比べる時は、塩分、水溫、溶存酸素量その他榮養塩類の垂直分布や含有量について、明かに季節的特徴を示していることがみられた。

水質に關しては特に可溶性有機物、燐酸塩、亞硝酸塩について、冬季と夏季とを比較して詳細に檢討を加えた。港內水の可溶性有機物は冬季に多く夏季の約1.4倍あり、底層の方が表層より含有量は多いが、層による差は小さい。これに反して夏季は表層が底層よりも多く且つ偏差も大きい。燐酸塩は夏季は冬季の1/5.2位であり、表層に少く底層に多いが、偏差は冬季の方が大きい。亞硝酸塩も冬季に多く夏季の約11.4倍である。夏季は底層が表層よりも多いが、冬季は夏季の逆の關係にある。窒素と燐との關係から考えると、室蘭港に於ては窒素が生產を支配する最小物質となつている樣に見える。

底質に關しては每回の調査結果がほゞ同樣ではあるが、鑛滓や船舶からの投棄物などにより部分的に粒度組成に變化を示している地點がみられた。

プランクトンは第4回の夏季に調査したのみである。その種類は大體同時期の噴火灣內のそれと共通であり、又採集時は所謂赤水のなかつた時であつたので、プランクトンによつて港內の汚染度を知ることは出來なかつた。

港內に棲息するホタテガイは泥量80%以下で港外水の影響をよくうける地域に多い。ホタテガイやアサリなどの棲息には底質の粒度組成以外に、浮游泥が直接間接に大きく影響を與えるものと考えられる。

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北寄貝肉の化學的研究

第 2 報　北寄貝水煮罐詰の黑變に就いて

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CHEMICAL STUDIES ON THE HOKKIGAI-MEAT (*Mactra sachalinensis* SCHRENGK)

II. ON THE BLACKENED MEAT OF CANNED HOKKI-GAI

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The author has studied on the blackened meat of the canned boiled Hokki-gai (*Mactra sachalinensis*), which was commercially processed. The mineral matters which are an important factor in the mechanism of black meat production, were separated by means of paper-strip chromatography.

The results obtained were as follows:

(1) In the black meat of the solid portion of the content, iron and copper were significantly recognized. A little lead also appeared. But no tin was seen at all.

(2) In the blackening liquid, iron and copper were significantly recognized, as much as lead. Trace of tin was recognized.

(3) In both the normal meat and liquid of the content, iron and copper were recognized. But lead and tin did not appear.

(4) In raw fresh shell-fish meat, both iron and copper were recognized too. But the amounts of these materials were considerablly less than in the canned products.

(5) Those mineral matters were considered to have found their way into canned products when raw meat was boiled in gun metal kettles, supposing from the results of a manufacturing test for rice boiler, in laboratory.

It is thought that the blackened meat was produced by binding of those mineral matter and hydrogen sulphide which were separated from the meat by boiling.

(6) The way to prevent such blackening of meat is to make use of a vessel for boiling which was prepared by painting its walls e.g., with C enamel, and to make no use of any gun-metal kettle.

カニ、アサリ等の軟體動物とか甲殼類等の肉を罐詰として製造する際、屢々肉質及び罐壁に黑變を生ずる現象は、既に多くの研究者により報告され、且つその病因についても研究されている。[1,2,3,4] 而してその主な生成機構は罐內容物がアルカリ性に於て、肉蛋白質を加熱する際生ずる硫化水素若くは硫化アンモンが肉質中の鐵或は罐より溶出する鐵と反應して硫化鐵を沈澱するものである。

本實驗に於ては、北寄貝を水煮罐詰として製造した際生じた黑變に就いて主としてペーパークロマトグラフ法により金屬の檢出を試み得られた結果を報告せんとするものである。

1. 罐詰原料及び製造工程

實驗材料として使用した罐詰の原料は昭和24年9月3日午後北海道長万部漁業會より荷受けしたものを翌9月4日午前より罐詰として製造した。此の間原料は洗滌槽に清水を滿水し、之に浸漬し放置した。又製造工程の概略を記せば次の如くである。

(Ⅰ) 調理: (1)脫殼(鐵製脫殼用庖丁を使用), (2)洗滌(洗い籠に適當量入れ輕く洗滌), (3)一番洗(ホーロー引又は亞鉛引バケツを以て剝身1個宛洗滌), (4)二番洗(一番洗と同樣), (5)三番洗, (6)潮吹口除去(庖丁を以て切斷する), (7)黑膜除去(外套膜周邊の黑膜を除去する), (8)内臟除去(指頭を以て內臟を除去する), (9)洗滌(1個宛清水により洗滌), (10)塩漬(洗籠に入れ Bé 5° の塩水に10分間塩漬し、終了後輕く洗滌する), (11)煮熟(Bé 5° の塩水中に3分間煮熟する。砲金製二重釜使用、塩漬終了後より煮熟迄の經過時間は大約40分であつた), (12)冷却(Bé 5° の塩水中に浸漬し、冷却する)。(Ⅱ) 肉詰: (13)肉詰(冷却後水切し肉詰する。肉詰量 165gm, 水切後肉詰迄の時間は色澤、香氣等に關する故迅速に實施した), (14)液汁注加(2回煮熟せる煮汁を2〜3回濾過し、之をホーロー引バケツに入れ冷却する。冷却後 Bé 3° の濃度に保ち、注入液として注加する。注入量 70gm.)。(Ⅲ) 脫氣, 卷締: (15)假卷締(肉詰完了後迅速に行う), (16)脫氣(180°F, 15分間), (17)本卷締, (18)殺菌(6Lbs. 80分間), (19)冷却(殺菌終了後洗滌槽中の清水に罐を浸漬し冷却する), (20)打檢(濁音罐を發見せず)。

2. 開 罐 狀 況

以上の如く製造せられた罐詰に就き昭和24年9月5日及び昭和24年10月26日の2回に分けて開罐試驗をした結果は次頁の第1表及び第2表に示す如くである。

第1表中Aの試料は特に北寄貝肉を鐵製釜を以て煮熟したものを用い、又注加液汁は砲金製二重釜により煮熟したものである。

3. 實 驗 の 部

(1) 試料の調製: 上記の如き罐詰を實驗試料となし、開罐後固形分及び液汁を分離し、固形分にあつては黑變部のみを採取し、夫々を電氣爐中に於て充分加熱し灰化した後、濃硝酸又は濃塩酸を約2cc加え、灰分をガスバーナー上で再び加熱し、殆ど乾固するまでになつた後、水及び 6-N 塩酸を加え溶解し、之を實驗に供した。同時に對照として黑變を生成せぬ北寄貝水煮罐詰の固形肉、液汁及び生鮮北寄貝肉を上記と同一方法により灰化し、實驗に供した。

(2) 實驗方法: 本實驗に於ては罐詰の黑變生成の因子として硫化水素による金屬元素の黑色沈澱を予測し、此の爲め銅、鐵、錫、鉛等が考えられ、之等を檢出する爲め Paper-strip-chromatography [(5)] に依つた。展開劑としては蒸溜水、アセトン－塩酸系、ブタノール－醋酸系、ブタノール－塩酸系溶媒を使用し、顯色試藥として硫化水素、若くは鐵及び銅は1% $K_4Fe(CN)_6$ 溶液、鉛は3%KI及び1% $KCrO_4$ 溶液を用いた。又同時に濾紙上に於て點滴反應を試みた。

Table 1. Result of the opening test of canned boiled Hokki–gai meat (Immediately after production)

Series	Total weight (gm)	Vacuum (Inch)	Weight of can (gm)	Weight of liquid (gm)	Weight of solid (gm)	Lot of solid	pH	Color (Solid)	Color (Liquid)	Odour	Refraction
A	284	10.5	63	94	127	3	6.2	No blackening on surface	Bluish blackening	Little of smell of shell–fish	9.5
B	284	10.3	61	98	125	3	6.4	Blackening on surface of shell–meat	Recognized bluish blackening precipitate	Little of odour of shell–fish	9
C	257	19	63	63	131	4	6.6	Blackening on surface of shell–meat	Recognized bluish blackening precipitate	Little of odour of shell–fish	7.5

Note: (1) There were no change in canned meat.

(2) It was bluish black in liquid. Recognized durk precipitate. This precipitate was produced on surface of canned meat. There was litte odour of shell–fish except of non putrescent oduor.

Table 2. Result of the opening test of canned boiled Hokki–gai meat.

NO. of sample	Total weight (gm)	Weight of solid (gm)	Amount of liquid (gm)	Vacuum (Inch)	Change of can wall	Surface of solid	Hardness of solid	Color of liquid	Taste of liquid	Remarks
1	215	75	75	16	Blackening at part of sealed frange.	No change	Normal	Blackening	Normal	pH 6.4
2	170	50	55	0	Blackening at part of frange and cover.	No change	Tender, a little of decompose.	Blackening	A bit of acid	pH 5.8
3	145	40	40	16	No change	Durk blackening	Normal	Blackening	Normal, salt	pH 6.5
4	140	40	35	16	No change	Durk blackening	Normal	Blackening	Acid	pH 6.5
5	165	45	55	0	Blackening at part of sealed frange.	Durk blackening	Normal	Blackening	Acid	pH 6.5
6	205	80	55	8	Blackening 2 parts of cover, a part on frange	No change	Hard	Blackening	Acid	Used parchment paper. pH 6.4
7	165	55	40	0	Blackening several parts of cover, half of frange.	No change	Tender	Blackening	Acid	Used parchment paper. pH 5.8
8	160	55	35	25	Blackening at several parts of cover, and frange.	Durk blackening	Normal	Little blackening	Normal	Used parchment paper. pH 6.5
9	155	60	25	18	No change	Durk blackening	Tender	No change	Bitter	Used parchmen paper. pH 6.5
10	160	50	40	16	Blackening at a part of cover, and several parts of body.	Durk blackening	Hard at several parts.	Little blackening	Acid	Used parchment paper. pH 6.5

(3) 實驗結果: 以上の方法に依り得られた結果は第3表, 第4表, 及び第1圖に示す如くである。

此の結果より明かな如く黑變部には鐵及び銅の反應が顯著であり、鉛も若干存在するのが認められたが錫は認められなかつた。而して黑變罐詰の液汁中には同じく鐵及び銅の反應は顯著であり、鉛もその存在することが認められた。又錫も液汁中に微量存在する如く見受けられた。對照とした良好罐詰の固形分中には鐵、銅の存在は認められたが鉛及び錫の存在は確認出來なかつた。此の液汁中に於ては固形分と同一の結果が得られた。生鮮肉中には銅及び微量の鐵が檢出されたが、鉛及び錫は認められなかつた。

Table 3. Result of spot reaction

Sample	Fe	Cu	Sn	Pb
Blackened meat of solid portion	+ $K_4Fe(CN)_6$	+ $K_4Fe(CN)_6$	− H_2S	+ KI + $KCrO_4$
Blackening liquid	+ $K_4Fe(CN)_6$	+ $K_4Fe(CN)_6$	− H_2S	+ KI
Normal canned meat	+ $K_4Fe(CN)_6$	± $K_4Fe(CN)_6$	− H_2S	+ KI
Normal liquid	+ $K_4Fe(CN)_6$	± $K_4Fe(CN)_6$	− H_2S	+ KI

4. 考　察

以上の事實を考察するに先づ軟體動物の生體中に存する無機成分に就いて既に多くの研究者により報告されている如く、(6)(7)(8) 鐵及び銅の存在が證明されている。北寄貝鮮肉中に於ても鐵及び銅の存在が認められ從つて罐詰とした場合、その內容物中固形分に鐵、銅の存在が認められるのは當然と思われる。更に罐詰に於ては罐自體より液

Table 4. Recognized mineral matters by means of paper-stip-chromatography.

Sample	Solvent: Butanol–HCl Fe(Rf)	Cu(Rf)	Sn(Rf)	Pb(Rf)	Aceton–HCl Fe(Rf)	Cu(Rf)	Sn(Rf)	Pb(Rf)
Blackened meat of solid portion	+ (0.55)	+ (0.25)	−	+ (0.15)	+ (0.80)	+ (0.70)	−	+ (0.30)
Blackening liquid	+ (0.50)	+ (0.25)	± (0.85)	+ (0.20)	+ (0.85)	+ (0.73)	± (0.90)	+ (0.32)
Normal canned meat	+ (0.55)	+ (0.25)	−	−	+ (0.82)	+ (0.70)	−	−
Normal liquid	+ (0.55)	+ (0.30)	−	−	+ (0.80)	+ (0.88)	−	−
Fresh raw shell-fish meat	+ (0.60)	+ (0.30)	−	−	+ (0.82)	+ (0.70)	−	−

Aceton–HCl: The solvent which were added to 10% of volume with conc. hydrochloric acid into aceton.

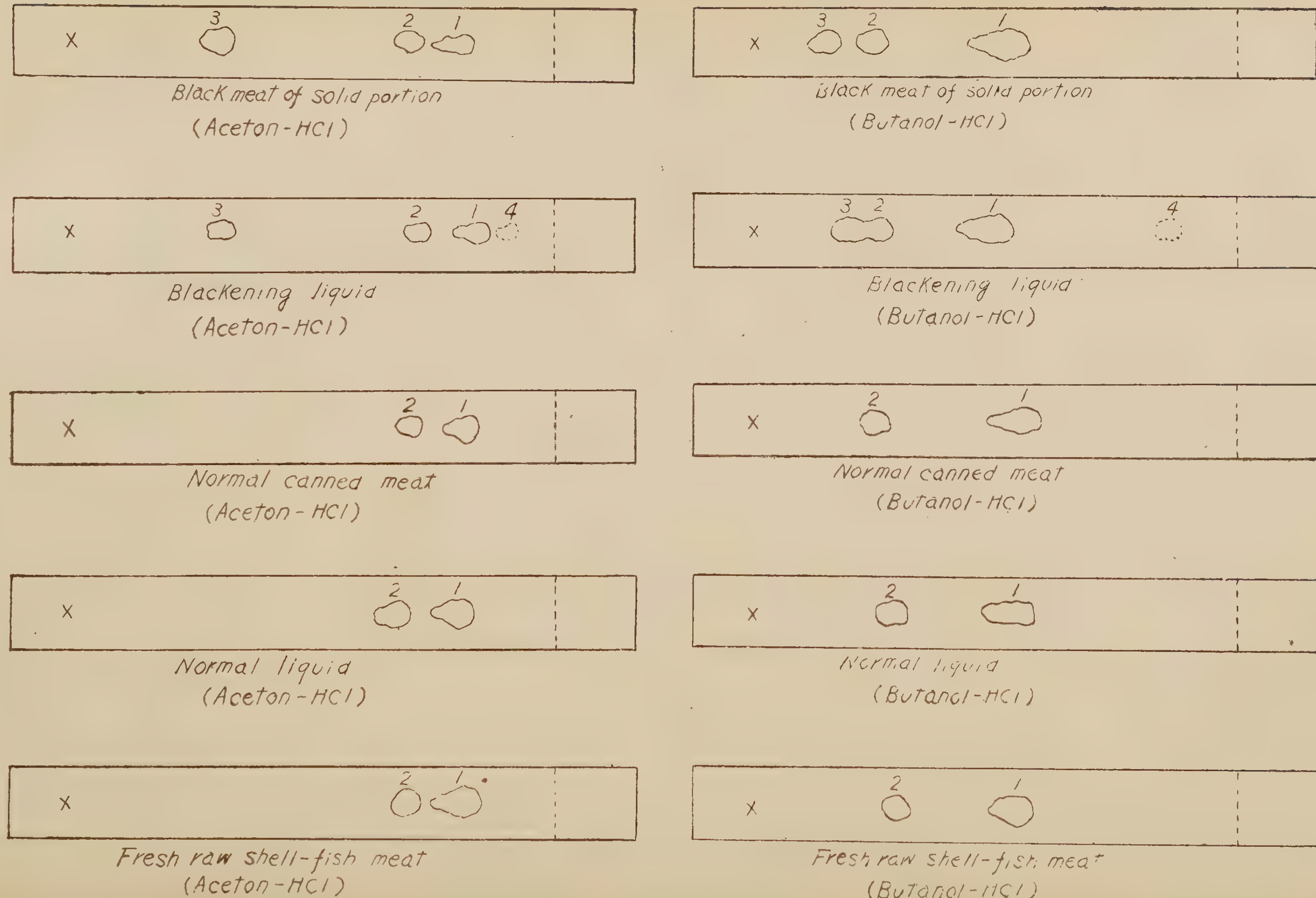
Butanol–acetic acid: Graciall acetic acid were added with 20% of volume into butanol. After addition of 70% of volume of water into the solution, only the part of butanol was used.

Butanol–HCl: The solvent which were added to about 50% of volume with 6N-hydrochloric acid into butanol.

Note:

1. For the use of butanol-HCl solvent, it was defficult to separate of iron and copper (temp. 23°C ascended at 20hrs).
2. The spot of iron was tailing.

Fig 1. Paper chromatograms of separated mineral matter. 1. Iron. 2. Copper. 3. Lead. 4. Tin.



汁中に溶出すると考えられる金属として、錫、鉛、鐵が存するが黒變を生成せぬ罐詰の液汁及び固形肉中に錫及び鉛が検出されず、又錫及び鉛より遙かに電化列の小である銅が反應を起していることよりみて、本實驗に使用した罐詰試料では罐自身より溶出する金屬はchromatographyにより檢出され得ないと云うものではなく、寧ろ罐詰より溶出する金屬は無視してもよいと思われる。勿論鐵はその電化列は上記三元素より遙かに上位に在るのであるが、罐詰内部では液汁に直接露出する部分は錫であり、從つて鐵の溶出は特殊な場合即ちpin holeの存在以外には考慮する必要はなく、事實亦開罐試験の結果よりしても罐壁の黒變は僅少であつて罐自身より内容物中に介入する鐵は無視出來るものの如く考えられる。然るに黒變を生成した罐内容物にあつては固形肉及び液汁共に鉛の存在を認め且つ液汁にあつては錫の痕跡をも認めた事實は興味に値する。

而して上記の金屬元素、殊に錫及び鉛が罐より溶出したものでなければ之は當然外部より齎されたものであると思われる。之等金屬が罐外より搬入せられる機會を考える場合、先づ製造工程中に於て之等の金屬元素を含有する器具を使用した場合、及び使用水中の金屬の存在が擧げられる。前者に於ては鐵製庖丁及び煮熟用鐵製二重釜、砲金製二重釜を使用し、後者は水道水を用いた。それ故後者によつては鉛及び錫が罐内に溶入する如きは考えられず、唯前者の場合のみが注意される。從つて煮熟に使用した鐵製二重釜、砲金製二重釜、及びホーロー引煮熟釜を用い、製造試驗を行つた結果を記せば次の第5表の如くである。

Table 5. Result of manufacturing test in laboratory-scale

Series of kettle	pH Solid	pH Liquid	Color of can contents
Gun-metal	6.0	6.5	Bluish blackening in liquid, as much as surface of solid.
Iron	6.2	6.4	No change. a bit of durkness in liquid
Enamelled ironware	6.2	6.3	No change.

以上の結果より明らかな如く、砲金製二重釜により煮熟した場合に限り黒變を生成し、從つてその生成の一因として砲金よりの金屬の溶出も考慮出來るのである。更に一般に砲金は銅90%, 錫10%の合金であつて、之等金屬元素は容易に遊離するものではないが、工場に於て使用したものは極めて粗悪な製品であり、第2圖に示す如く内面各所に痕渣を生じて居り、鉛を以て充塡したものである。尙此の二重釜を放置すれば綠銹を生じ、從つて銅の遊離も考えられる。

Fig 2. Inner part of gun-metal kettle



それ故特に砲金製二重釜を使用し、水道水に關する試驗及び北寄貝罐詰注入液の製造試驗を行い、次の第6表及び第7表の如き結果を得た。

上記の事實より本實驗に使用した黒變罐詰中の鉛及び錫は罐詰製造時に使用した砲金製二重釜より遊離したものゝ如く解される。而して錫の痕跡が黒變液汁中のみに見受けられたのは固形分の煮熟が比較的短小であるのに對し、注加液の加熱時間が長時間に亘つた爲めであろう。又黒變を生成する機作に關しては、溶出した錫、鉛等の金屬元素と肉蛋白を加熱した際生成した硫化水素が作用し黒變を生じたものと解される。同時に又、北寄貝よりの鐵及び銅も此の場合對照とした通常の北寄貝水煮罐詰の固形部及び液汁に鐵及び銅の反應が認められるに拘らず黒變を生成せぬ點

から推して北寄貝より溶出する鐵及び銅は反應せぬものゝ如く推定される。此の點に關しては尚兩者の量的な關係を追究する必要がある。

Table 6. Result of heating of tap-water in gun-metal kettle

Temp. (℃)	pH	S.G.	Odour	Color
15℃	6.3	1.008	Non	Non
50	6.8	—	Little of metal-odour	Non
80	6.8	—	Metal-odour	Non
98	7.0	—	Much of metal-odour	Non

Table 7. Result of manufacturing test of liquid.

Series of kettle	pH	Odour	Color
Iron	6.3	Normal	White
Gun-metal	6.5	Weakening of shell-meat oduor	A little of blackening

又肉蛋白を加熱する際生成する硫化水素の發生量は加熱溫度時間、及び pH により變化する事は既に知られているのであるが、開罐時の結果よりも明らかな如くその pH は概して酸性側に在ることよりしても研究の對照とした黒變が從來提起された問題と幾分その性質を異にするものゝ如く思われる。肉蛋白の加熱時間が長く、又溫度が高ければ硫化水素の發生量も大となる故、製造工程中北寄貝を煮熟する場合、溫度及び時間に留意せねばならぬことも勿論必要と思われるが、之に關しても更に實驗する必要がある。

以上の如く生成する黒變の防止策としては、先づその生成因子が罐外より介入したものである故に製造工程を再檢討すべきであり、例えば調理工程中、可及的金屬元素を含有する如き器具の使用を避け、又罐詰を卷締める際硫化水素の發生を阻止する目的を以て罐內容物の pH を低下せしめる爲め、炭酸、枸櫞酸、乳酸若くは酒石酸の如き酸を用いて酸性とするのが望ましく、又罐自體より溶出する錫、鐵、鉛等による黒變の防止には廣く使用される內面塗料罐例えば c エナメル罐、ラツカー罐等の使用が良いと思われる。

5. 要　　約

北寄貝を水煮罐詰として製造した際生じた黒變に就き研究した。肉蛋白を加熱する際發生する硫化水素と反應して黒色沈澱を生ずる金屬を主として Paper-strip-chromatography により分離檢出を試み、得られた結果を要約すれば次の如くである。

(1) 罐內容物中、固形部にある黒變部では、鐵及び銅が顯著に認められた。又少量の鉛の存在も認められたが錫は檢出出來なかつた。

(2) 黒變した液汁中には鐵及び銅が顯著に認められ、鉛も亦その存在が認められた。又錫の痕跡も存在する如く見受けられた。

(3) 黒變を生成せぬ白色肉及びその液汁中に於ては、鐵及び銅の反應が認められたが、鉛及び錫は認められなかつた。

(4) 對照として使用した新鮮北寄貝肉中に於ては銅の存在が認められたが、之等は量的にみて罐詰製品とされたものより低い値を示す如く思われた。錫及び鉛の存在は何れも認められなかつた。

(5) 製造工場に於て實際使用した煮熟用釜を以て製造試験を行つた結果より、之等の鐵、銅、鉛、錫等の金屬の一部は砲金製二重釜より遊離し、罐詰製品中に介入せるものゝ如く思われ、而して之等金屬が北寄貝肉を煮熟する際生ずる硫化水素と反應して黑變を生ずるものと推定される。

(6) 黑變肉生成の防止策としては廣く一般に用いられている內面塗料罐を施した罐、例えばcエナメル罐、ラツカー罐を使用すると共に、斯の如き砲金製二重釜の使用を可及的忌避すべきである。

本實驗の實施に際し、終始御懇篤なる指導及び本稿の校閱を賜わつた本學部谷川英一教授、秋場稔講師、並びに實驗に協力された研究室の諸氏に對し深甚なる謝意を表する。

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